Final Review Report and Regulatory Decision

Australian Pesticides & Veterinary Medicines Authority

The reconsideration of registrations of products containing dimetridazole and their associated approved labels

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FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the *Agricultural and Veterinary Chemicals Code Act 1994* (Agvet Code).

The APVMA can reconsider the approval of active constituents, the registration of chemical products or the approval of labels for containers of chemical products at any time. This is outlined in Part 2, Division 4 of the Agvet Code.

The basis for the reconsideration is whether the APVMA is satisfied that continued use of products containing dimetridazole in accordance with the instructions for their use:

- would not be an undue hazard to the safety of people exposed to them during their handling
- would not be likely to have an effect that is harmful to human beings.

The APVMA also considered whether the use of products containing dimetridazole in accordance with the instructions for use that the APVMA has approved would be effective according to the criteria demanded by the APVMA for the products.

The requirement for continued approval of a label for containers for a chemical product is that the label contains adequate instructions. Such instructions include:

- the circumstances in which the product should be used
- how the product should be used
- times when the product should be used
- frequency of the use of the product
- the withholding period after the use of the product
- disposal of the product and its container
- safe handling of the product.

A reconsideration may be initiated when new research or evidence has raised concerns about the use or safety of a particular chemical, a product or its label.

The process for reconsideration includes a call for information from a variety of sources, a review of that information, and following public consultation, a decision about the future use of the chemical or product.

In undertaking reviews, the APVMA works in close cooperation with advisory agencies including the Office of Chemical Safety (OCS), the Department of Environment and Water Resources, and state departments of agriculture as well as other expert advisors as appropriate.

The APVMA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of review reports is part of that process.

The APVMA also makes these reports available to the regulatory agencies of other countries as part of bilateral agreements or as part of the Organisation for Economic Cooperation and Development (OECD) *ad hoc* exchange program. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the original data from the relevant applicant.

This document, *The reconsideration of registrations of products containing dimetridazole and approvals of their associated labels*: *Final Review Report and Regulatory Decision,* relates to all products containing dimetridazole that were nominated for review by the APVMA. The review's findings and regulatory decision are based on information collected from a variety of sources, including data packages and information submitted by the registrants, information submitted by members of the public including users/industry groups and government organisations, and literature searches. The information and technical data required by the APVMA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken.

The *Final Review Report and Regulatory Decision* is available from the APVMA website: http://www.apvma.gov.au/chemrev/chemrev.html.

GLOSSARY

Time		Weight	
d	day	**bw**	body weight
h	hour	**g**	gram
min	minute	**kg**	kilogram
mo	month	**µg**	microgram
wk	week	**mg**	milligram
s	second	**ng**	nanogram
yr	year	**wt**	weight

Length		Dosing	
cm	centimetre	**id**	intradermal
m	metre	**im**	intramuscular
µm	micrometre	**inh**	inhalation
mm	millimetre	**ip**	intraperitoneal
nm	nanometre	**iv**	intravenous
		po	oral
		sc	subcutaneous
		mg/kg bw/d	mg/kg bodyweight/day
		w/w	weight/weight

Volume		Concentration	
L	litre	**M**	Molar
mL	millilitre	**ppb**	parts per billion
µL	microlitre	**ppm**	parts per million

Chemistry	
HPLC	High Pressure Liquid Chromatography

Terminology	
ADI	Acceptable Daily Intake
Agvet Code	*Agricultural and Veterinary Chemicals Code Act 1994*
ARfD	Acute Reference Dose
LOD	Limit of Detection
LOQ	Limit of Quantitation
LOEL	Lowest Observed Effect Level
MRL	Maximum Residue Limit
NOEL	No Observed Effect Level
OHS	Occupational Health and Safety
SD	Sprague Dawley
WHP	Withholding period

Organisations & publications

APVMA	Australian Pesticides and Veterinary Medicines Authority (previously NRA)
EMEA	European Agency for the Evaluation of Medicinal Products
EU	European Union
FAO	Food and Agriculture Organization (of the United Nations)
FAISD Handbook	Handbook of First Aid Instructions and Safety Directions
JECFA	FAO/WHO Joint Expert Committee on Food Additives
NHMRC	National Health and Medical Research Council
NOHSC	National Occupational Health and Safety Commission
NRA	National Registration Authority for Agricultural and Veterinary Chemicals (now APVMA)
OCS	Office of Chemical Safety
OECD	Organisation for Economic Cooperation and Development
PACC	Australian Pesticides and Agricultural Chemicals Committee
PACSC	Pesticides and Agricultural Chemicals Standing Committee of the NHMRC
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
US EPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
WHO	World Health Organization

Poultry industry terminology

Breeder chicken hen	Alternate term for female broiler breeder
Breeder hen	Refers either to a breeder chicken hen or breeder turkey hen
Breeder turkey hen	Alternate term for female turkey breeder
Breeder poultry	Refers in this document only to broiler breeder, layer breeders and turkey breeders
Broiler chicken	Young chicken fattened to produce meat for human consumption
Broiler breeder	Chicken hen that lays fertilised eggs from which chickens for fattening are hatched. Males fertilise the eggs.
Broiler/Grower (meat) turkey	Young turkey fattened to produce meat for human consumption
Chicken layer hen	Chicken that produces table eggs for human consumption
Layer breeder	Chicken hen that lays fertilised eggs from which chickens reared for table egg production are hatched. Males fertilise the eggs.
Poultry	Collective term representing birds that produce meat and eggs for human consumption
Replacement pullet	Immature male or female chicken. Females are reared to produce table eggs for human consumption or fertilised eggs for hatching
Replacement poult	Immature male or female turkey. Females are reared to produce table eggs for human consumption or fertilised eggs for hatching
Squab	Young pigeon reared for meat production for human consumption
Turkey breeder	Turkey hen that lays fertilised eggs from which turkeys for fattening are hatched. Males (toms) fertilise the eggs.
Turkey layer hen	Turkey that produces table eggs for human consumption

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EXECUTIVE SUMMARY

Introduction

Products containing dimetridazole are currently approved in Australia for use in animals to treat and prevent diseases caused by certain infectious organisms. Dimetridazole is currently approved for the treatment and prevention of 'blackhead' in poultry, and 'canker' in pigeons and caged birds. Blackhead is caused by a protozoan species, *Histomonas meleagridis.* Turkeys are particularly susceptible to *H. meleagridis* infection. Canker is caused by another protozoan species, *Trichomonas gallinae.* Dimetridazole is also approved for the prevention of swine dysentery in pigs caused by the bacterial species, *Brachyspira hyodysenteriae*.

Registrations and label approvals for products containing dimetridazole are being reviewed as part of the APVMA's Review Program. The review was initiated in July 2002 because dimetridazole has been withdrawn from use in food-producing animals in several countries, primarily due to unresolved concerns regarding its potential carcinogenicity and uncertainty surrounding the longevity of residues in treated animals. Published reports have indicated that there is a potential for human exposure to residues of dimetridazole in food following the treatment of food-producing animals.

In this review the APVMA, in collaboration with its advisory agencies, has completed an assessment of data from the registrants, public submissions, scientific literature, archival holdings and reviews by overseas regulatory authorities.

Public submissions

Twenty-one written submissions were received from state government departments, registrants and user groups in response to the initial data call-in and the draft review report. The concerns outlined in the submissions included:

- repeated observations that there is no suitable alternative chemical treatment available to control blackhead in poultry (particularly turkeys)
- the potential for an adverse economic impact on a range of business interests arising from any decision to restrict the use of the chemical
- the potential negative impact on health, production and welfare of valuable breeding pigs and poultry
- the potential impact on human health, including worker safety particularly at the level of the end user, associated with the use of a chemical which is a possible carcinogen.

The concerns as outlined in the submissions received were considered in the review.

Registrants' submissions

There were no new toxicological studies submitted by the registrants as a result of the data call-in. Many of the archived toxicological studies date back to the 1960s, and in some instances to the late 1950s, and therefore do not conform to current test guidelines or levels of reporting.

Toxicology assessment

The review reaffirmed the incompleteness of the toxicology database and finds that:

- no significant addition to the toxicology database has occurred since the evaluation in 1986 to adequately address the issue of dimetridazole potential for carcinogenicity
- data on metabolism of dimetridazole in turkeys and pigs are referenced in this review but no data on metabolism of dimetridazole in laboratory animals were submitted to the review. Such data are crucial in determining the relevance of animal models used in toxicity studies to address the potential toxicity of dimetridazole residues in humans
- for a number of toxicology studies, only summary reports of studies were available for evaluation
- experimental deficiencies in a number of toxicity studies, such as the use of only a small number of animals and/or monitoring of only a few study parameters, limited the regulatory usefulness of the studies
- no observed effect levels (NOELs) for toxic endpoints could not reliably be set in many toxicity studies (including chronic, reproductive and developmental toxicity studies) because of the absence of sufficient study details or deficiencies in study design
- dimetridazole's potential to cause carcinogenicity and developmental effects in a second laboratory animal species was not assessed by OCS because of the absence of such studies although data from reproductive studies in pigs were considered in 1986 by the Australian Pesticides and Agricultural Chemicals Committee (PACC)
- in a 'comet' assay conducted in mammalian cells, dimetridazole showed evidence of genotoxic potential
- although dimetridazole did not show genotoxic effects in available *in vivo* studies, some related 5-nitroimidazoles (eg metronidazole, ronidazole) are genotoxic.

As a result of the toxicology assessment, this review finds that these deficiencies are significant and accepts the advice of the Office of Chemical Safety (OCS) that an acceptable daily intake (ADI) can no longer be supported. Since the concerns about dimetridazole were not allayed, it is not safe to use dimetridazole in food-producing species. Therefore, the APVMA cannot be satisfied that the continued use of dimetridazole in food-producing species, such as pigs, chickens and turkeys would not be likely to have an effect that is harmful to human beings.

Without an ADI for dimetridazole, the APVMA cannot conduct a risk assessment to determine a safe consumption level of dimetridazole in food commodities. Without knowing what would be a safe consumption level of dimetridazole in food commodities, the APVMA cannot vary the conditions of registration and label approval of the products which have uses only in food-producing species such that the requirements for their continued registration will be complied with, and the criteria for continued label approval are met.

Nevertheless, from a toxicology perspective, the continued availability of dimetridazole products for the treatment of companion animals and birds not destined for human food can be supported.

Occupational health and safety assessment

The data call-in notifications provided to registrants and other stakeholders primarily requested the provision of toxicological data in order to conduct a hazard assessment. However, the scope of the current review included the proviso that 'depending on the outcome of the toxicological assessment, an assessment of the occupational health and safety, food residues and trade issues may be required'.

Since no suitable toxicological data were submitted, there were insufficient data on which to reliably establish effect levels to permit an occupational health and safety risk assessment to be modelled. Consequently, in the absence of suitable toxicological data, a qualitative risk assessment was undertaken to identify suitable exposure control/mitigation measures for workers and safety directions for end users in the event that uses of dimetridazole in pigeons, caged birds and game birds were to be permitted.

The review found that the greatest risk of exposure to dimetridazole is likely to occur in feed milling processes, on-farm mixing and during transportation where dermal and inhalation (dust) exposures to existing products are possible. In order to reduce the risk from acute and repeated exposures, the use of personal protective equipment was recommended and new safety directions are to be observed.

The APVMA is satisfied that the continued use of dimetridazole in non-food-producing species in accordance with the varied label instructions, would not be an undue hazard to the safety of people exposed to it during its handling or use for the indications which will remain.

Residues assessment

The APVMA's Chemistry and Residues Program undertook a residues assessment based on existing available residues data for poultry. This assessment took into account the possibility that excess eggs from breeder chickens and breeder turkeys and meat from culled breeder replacement pullets might enter the human food chain.

The Chemistry and Residues Program investigated the feasibility of dimetridazole being permitted for use in breeder turkeys and breeder chickens for controlling blackhead. The rationale at the time of the initial risk assessment was that the use of dimetridazole in breeder poultry could be justified from a human health perspective, if the withholding period was lengthened to ensure that there were no dimetridazole residues in excess eggs and meat from culled or spent breeder birds, which could enter the human food supply. The same rationale was applied in considering the use of dimetridazole in pigs for eradicating swine dysentery. Maximum Residue Limits (MRLs) under this rationale were determined at or about the limit of quantitation (LOQ) for the analytical method in place at the time that most of the dimetridazole studies were conducted.

However, it was subsequently realised that under current APVMA and international risk assessment methodology, it is invalid to use the method LOQ as a pseudo health standard, as the LOQ has no correlation to any toxicological endpoint used to establish an ADI or an acute reference dose (ARfD). In the absence of an ADI or ARfD for dimetridazole, the APVMA is not able to conduct an acute or chronic dietary intake risk assessment for dimetridazole residues in foodstuffs and hence to establish a safe consumption level for dimetridazole. Therefore, consumption of commodities containing residues, even at levels below the method LOQ, may constitute an undue risk

to human health. Thus, the APVMA is not satisfied that the continued use of dimetridazole in food-producing species, such as pigs, chickens and turkeys would not be likely to have an effect that is harmful to human beings.

The review finds that, from a residues perspective:

- the residue definition for dimetridazole will be amended to include the parent compound and its hydroxy metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole
- the continued use of dimetridazole in breeder chickens, breeder turkeys and pigs was not supported
- the potential for dimetridazole being used off-label in food-producing species as a result of its on-going availability for use in pigeons, caged birds and game birds may constitute a risk to human health if the chemical is not restricted from being administered to birds destined for human consumption.

For the purpose of a phase-out period the APVMA decided:

- to reduce the existing MRLs of dimetridazole for poultry meat and edible offal from *0.005 mg/kg to *0.0001 mg/kg, and to establish an MRL of *0.0001 mg/kg for eggs
- to increase the withholding periods for pig meat and poultry meat and eggs from five days to 28 days, and to establish a re-treatment interval of 28 days after the last treatment period for breeder chickens, breeder turkeys and breeder pigs
- to include an instruction on product labels that restrains users from administering dimetridazole to laying hens, broiler chickens and meat turkeys.

Adequacy of label instructions

The review identified some deficiencies in current label instructions. The labels do not provide adequate instruction on the hazard potential of products containing dimetridazole, nor do they include any OHS exposure minimisation instructions. There are also inconsistencies between label withholding period statements. For cancelled products, the APVMA decided to cancel all existing labels and to issue new instructions on how the products are to be handled and used during a phase-out period. The APVMA also decided to vary the labels for products whose registrations can be affirmed, with new instructions relating to the use of dimetridazole in pigeons, caged birds and game birds.

Conclusions

This review concluded that the registrations of products, for which on-going use of dimetridazole in non-food-producing animals is supported, could be affirmed after the product labels are varied by the inclusion of new instructions. This review further concluded that since it is not safe to use dimetridazole in food-producing species, the registrations of products currently used in food-producing animals could be supported.

Summary of review recommendations

Based on the assessments in this review, the APVMA decided to:

- cancel the registration of products that contain dimetridazole for use only in food-producing-species
- cancel label approvals of these products
- affirm the registration of products with labels that can be varied such that they are only for use in non-food-producing-species
- amend the residues definition of dimetridazole to the sum of the parent compound and its hydroxy metabolite
- phase out the cancelled uses of dimetridazole in 24 months, and
- vary labels for products which will remain in the market to include amended safety directions and a warning statement indicating that dimetridazole may cause genetic damage in users.

For the duration of the phase-out period for products which have uses only in food-producing animals, the APVMA further decided to:

- issue new instructions on how the cancelled products are to be handled and used
- establish new temporary MRLs for poultry meat, pig meat, edible offal and poultry eggs
- establish a withholding period of 28 days for meat and eggs
- establish a re-treatment interval of 28 days for breeder chickens, breeder turkeys and breeder pigs.

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1. INTRODUCTION

1.1. Regulatory status of dimetridazole in Australia

Dimetridazole is a derivative of 5-nitroimidazole. The systemic name is 1,2-dimethyl-5-nitro-1H-imidazole ($C_5H_7N_3O_2$). There are other 5-nitroimidazoles currently registered for use in humans and animals in Australia, generally for the treatment and prevention of protozoal infections. There are few alternative chemical treatments available for this purpose.

Products containing dimetridazole are currently registered for use in pigs, poultry, turkeys, game birds, pigeons and other caged birds. When this review commenced in July 2002, there were four registrants of seven veterinary products. The registration of one of these products has since not been renewed (refer to Appendix B for the list of registered products). The permitted uses for products containing dimetridazole are:

- treatment and prevention of blackhead (caused by *Histomonas meleagridis*) in game birds, poultry and other caged birds
- treatment and prevention of swine dysentery (caused by *Brachyspira hyodysenteriae*) in pigs
- treatment of canker (caused by *Trichomonas gallinae*) in pigeons and caged birds.

Products containing dimetridazole can be applied as in-feed medication and/or by addition to drinking water.

No adverse effects associated with the use of any product containing dimetridazole have been reported to date to the APVMA's Adverse Experience Reporting Program.

1.2. Reasons for the review of dimetridazole

The decision to review products containing dimetridazole stems from concerns over human health.

The United States Environmental Protection Agency (US EPA) withdrew approvals for dimetridazole on the basis of its mutagenicity, and suspected carcinogenicity of its metabolites, but maintained some uses under veterinary prescription in non-food-producing animals.

The European Union (EU) also withdrew authorisation for the use of dimetridazole as a veterinary medicine over concerns about its potential carcinogenicity, whereas in Canada, the use of all 5-nitroimidazole chemicals in animals that produce food for human consumption has been prohibited due to a lack of toxicological information on bound residues of metabolites.

Moreover, the Joint Expert Committee on Food Additives (JECFA) reported that insufficient information is available to support the setting of an ADI or MRL for dimetridazole and later declared that dimetridazole is mutagenic *in vitro*. Details of these regulatory decisions are given in Section 6, Overseas Regulatory Status.

1.3. Scope of the review

In July 2002 the APVMA commenced the review of registrations and approvals relating to dimetridazole by releasing a scope document which made specific reference to concerns over the possible carcinogenicity of the chemical.

The basis for a review of the registration and approvals for a chemical is whether the APVMA is satisfied that the requirements prescribed by the Agvet Code for continued registration and approval are being met. In the case of dimetridazole the relevant requirements were that the use of products containing the chemical in accordance with the instructions for their use would not be likely to have an effect that is harmful to human beings and would not be an undue hazard to the safety of people exposed to them during handling.

The July 2002 Review Scope Document stated that the review would be limited to the toxicological aspects of dimetridazole, but depending on the outcome of the toxicological assessment, an assessment of issues related to occupational health and safety (OHS), food residues and trade may be required.

The requirement for product labels prescribed in the Agvet Code is that the label contains adequate instructions. Such instructions include:

- the circumstances in which the product should be used
- how the product should be used
- times when the product should be used
- frequency of the use of the product
- the withholding period after the use of the product
- disposal of the product and its container
- safe handling of the product.

The July 2002 Review Scope Document canvassed an assessment of the adequacy of label instructions on current approved labels for products containing dimetridazole.

1.4. Regulatory options

In general there can be three possible outcomes to the review of the registration of products and their labels. Based on the information reviewed, the APVMA may:

- be satisfied that the products and their labels continue to meet the prescribed requirements for registration and approval and therefore confirm the registrations and approvals
- be satisfied that the conditions to which the registration or approval is currently subject can be varied in such a way that the requirements for continued registration and approval will be complied with and therefore vary the conditions of registration or approval
- not be satisfied that the requirements for continued registration and approval continue to be met and thus suspend or cancel the registrations and/or approvals.

2. TOXICOLOGY ASSESSMENT

The APVMA commissioned a report from the Office of Chemical Safety (OCS) in the Therapeutic Goods Administration (TGA). OCS re-evaluated previously submitted studies, evaluated newly submitted data, and considered some evaluations completed by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1990. The following paragraphs in this section (2.1 to 2.6) are extracted from the OCS report.

2.1. Introduction

Dimetridazole belongs to a class of 5-nitroimidazoles, some of which are used to treat protozoal and bacterial (anaerobic) diseases in man and other animals. Structurally related compounds include metronidazole, tinidazole, nimorazole, ronidazole, ipronidazole, ornidazole and benznidazole.

In Australia, dimetridazole is one of two 5-nitroimidazoles registered for use in veterinary medicine. There are currently six registered products. Two products are pure dimetridazole powder (water insoluble), two products are water-soluble powders and the remaining two are premixed feed supplements. A table of registered products is at Appendix B.

The permitted uses of the products in Australia are treatment and prevention of:

- swine dysentery caused by *Brachyspira hyodysenteriae*
- blackhead in chickens, turkeys and game birds caused by *Histomonas meleagridis*
- trichomoniasis (canker) in pigeons and caged birds caused by *Trichomonas gallinae.*

Products containing dimetridazole may be applied as an in-feed medication and/or addition to drinking water. For disease prevention, labels recommend either continuous or regular feeding, while for treatment, dosing for between three and 14 days is generally recommended. Currently all products have a five-day withholding period for slaughter. The use of dimetridazole products in poultry producing eggs for human consumption is not permitted.

Dimetridazole is listed in Schedule 4 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). It has an Acceptable Daily Intake (ADI) of 0.002 mg/kg bw/d (set in 1986) based on a NOEL of 3.8 mg/kg bw/d in a two-year rat dietary study and a 2,000-fold safety factor. The large safety factor was applied because the database on dimetridazole was incomplete.

Dimetridazole has received particular attention from regulatory authorities in the Australian Government Department of Health and Ageing, including OCS, because of concerns over its possible carcinogenic and mutagenic effects. In 1988, the United States announced the withdrawal of approval for dimetridazole over concerns relating to the potential carcinogenicity of residues. In response to these concerns, in 1990 the Australian Pesticides and Agricultural Chemicals Committee (PACC) evaluated an updated toxicology and residue package, and noted an increased incidence of benign mammary tumours in both sexes in a chronic dietary study in rats. The tumours occurred earlier in treated animals than in controls, with multiple tumours in affected rats. The PACC stated that there appeared to be a clear dose above which the compound exerted its effect (a threshold level) and thus suggested that it was unlikely to be a

genotoxic carcinogen. However, in order to reduce the possibility of human dietary exposure to dimetridazole, the committee recommended its use in poultry be limited to breeders. The committee also recommended that a Maximum Residue Level (MRL) be set at the limit of detection in pigs and poultry.

In 1990 JECFA evaluated the database for dimetridazole but could not establish an ADI for dimetridazole because of the absence of a carcinogenicity study in a second species.

In 1995 the European Union (EU) withdrew the authorisation of dimetridazole as a veterinary medicine over concerns regarding its carcinogenicity. In 1997 and 1999, Germany and Sweden respectively requested that the EU cancel the use of dimetridazole as a feed additive due to concerns over residues persisting beyond the withholding period and concern that it was a suspected genotoxic carcinogen. The EU's Scientific Committee on Animal Health in 2,000 concluded that dimetridazole was not a genotoxic carcinogen in mammals. This committee set a NOEL of 4.6 mg/kg bw/d and an ADI of 0.0046 mg/kg bw based on a 122-week rat study as well as a 1,000-fold safety factor. However, the EU and Canada, in 2001 and 2002 respectively, withdrew the authorisation of dimetridazole as a feed additive on the grounds that insufficient data had been submitted for re-evaluation.

2.2. Metabolism and toxicokinetics

The registrants of dimetridazole have not provided data to the APVMA on metabolism and toxicokinetics of dimetridazole in laboratory animals or in humans. In a rat study evaluated only by JECFA, it was noted that qualitatively similar metabolism was seen in rats and pigs. Metabolism via oxidation at the 2-methyl group and degradation of the nitroimidazole ring was common in these species (Heijbroek, 1976; cf. JECFA evaluation, 1990).

The 5-nitroimidazoles have been reported to undergo extensive metabolism. The biotransformation generally includes oxidation or reduction, with the ring structure remaining intact followed by scission of the ring to form an oxamic acid derivative (Koch and Goldman, 1979).

In turkeys (Law *et al.*, 1962; 1963), ~90% of a radiolabelled dose (32 mg/kg bw of ^{14}C-dimetridazole, po) was excreted in urine, faeces and expired air within three days. No detectable levels (limit of detection = 0.03 µg/g tissue) were found in tissues of the turkeys three days after the last dose. Extensive metabolism was seen and the major metabolites included 1-methyl-5-nitroimidazol-2-yl methyl hydrogen sulphate (44% of the metabolites) and 1-methyl-5-nitroimidazol-2-yl methyl carboxylic acid (MNICA, 26%). The minor metabolites were 2-hydroxylmethyl-1-methyl-5-nitroimidazole (HMMNI, ~10%), a conjugated glucuronide and two unidentified non-nitro metabolites (<10%).

In pigs (Unsworth, 1972; Mulcock and Unsworth, 1973a, b), total excretion after oral administration of ^{14}C-dimetridazole (28 mg/kg/bw) in urine, faeces and expired air was 62% and 76% (of the administered dose) at two and seven days post-dose, respectively. Seven days after dimetridazole administration, tissue residues (up to 1 ppm) were found in several tissues including the liver, kidney, spleen, fat and muscle. Only ~50% of the metabolites of dimetridazole were identified. The identified metabolites (in urine or as residues in tissues) included HMMNI and MNICA, but there were no conjugated metabolites.

2.2.1. Acute studies

Dimetridazole exhibited low oral acute toxicity, with an LD_{50} of 1,790 mg/kg bw in mice and 1,600 mg/kg bw in rats. Clinical signs of toxicity in both rats and mice included sedation, and mortality was caused by respiratory arrest (Hood, 1962b). Dimetridazole appears to be a non-irritant on the skin or eyes (although in another study, dimetridazole was reported to be 'a very mild irritant'), but sufficient details were not provided in the studies conducted (Cosar, 1957; Hood, 1962c).

The acute iv LD_{50} in mice was 290 mg/kg bw (Hood, 1962b). Rats treated with dimetridazole at 5,000, 10,000 and 20,000 mg/m^3 by inhalation for four hours showed signs of haemorrhage in lungs and cervical lymph nodes (Pullinger, 1976).

One product, Emtryl Soluble, containing 40% w/w dimetridazole showed an oral LD_{50} of 1,700 and 2,500 mg/kg bw/d in mice and rats respectively. The iv LD_{50} values were 60 and 70 mg/kg in mice and rats respectively (Hood, 1962b). Another product, Emtryl Premix, (22.5% dimetridazole) appears not to be a skin irritant (Hood, 1962c).

There were no other studies on the acute toxicity of dimetridazole or its products by the dermal route. The potential for skin sensitisation by dimetridazole or its products was also not fully investigated. Hood (1962c) observed no irritation in rabbits from Emtryl Soluble, but sufficient experimental details were not provided for OCS to assess this finding.

2.2.2. Short-term repeat-dose studies

Mice were given dimetridazole by gavage at doses of 100, 250, 500 or 1,000 mg/kg bw/d for five days (no control group). All animals in the highest dose group died. Parameters other than mortality were not recorded or reported (Cosar, 1957).

In a four-week study in rats given 50 or 100 mg/kg bw/d (by gavage) there were no treatment-related effects on clinical signs, haematology, clinical chemistry or histology (Cosar *et al.*, 1957).

In a dose-range finding study in dogs (1/sex/group, no control group), diets containing 0.36% or 1.08% of dimetridazole (~90 or 270 mg/kg bw/d) were fed to the animals for four weeks. Results submitted in summary form indicated that food consumption was markedly reduced in dogs of the 1.08% group when compared to the 0.36% group. The animals in the 1.08% group exhibited ataxia, which appeared to be more prominent in the hindquarters. Histological changes in the 1.08% group included petechial haemorrhages and nephrosis of kidney, haemorrhages of heart and spleen, central lobular cirrhosis and haemorrhages of liver. The lungs showed a proliferation of interstitial tissue, which reduced the air space area. Kidneys of animals from both groups showed cloudy swelling in the cells lining convoluted tubules and tubules comprising the medullary ray.

Mild atrophy of the seminiferous tubules with no mature spermatocytes present and moderate degeneration of spermatids were also noted in the testes of the male dog from the 0.36% group. The study (Salsbury Laboratories, 1962b; study summary was evaluated by JECFA, 1990) was not evaluated by the Australian Government Department of Health (since November 2001, the Department of Health and Ageing).

According to an earlier evaluation report by the Department of Health (1986), in dogs (2/dose) given dimetridazole at 50 or 100 mg/kg bw/d (presumably by the oral route), significant ataxia developed after 10–30 days of treatment, caused by micro-haemorrhages in the grey matter of the spinal chord. There was no damage to the neurones, which accounted for the regression of symptoms when the treatment had stopped. Haematology was normal.

2.2.3. Subchronic studies

Diets containing 0, 0.2, 0.4, 0.6, 0.8 or 1% of dimetridazole (approximately equivalent to 0, 200, 400, 600, 800 or 1,000 mg/kg bw/d) were fed to rats for 13 weeks. Results from the study were submitted only in summary form. Deaths (females only) were seen in the highest dose group. Clinical signs included ataxia, tilted head, anaemic appearance, excitation and convulsions in the animals which died. Data for bodyweight, food consumption or dimetridazole intake were not provided.

Males dosed with 0.8% and 1% dimetridazole had albumin in the urine. Histopathology revealed testicular atrophy or degeneration in all treated groups. Severe atrophy of seminiferous tubules and absence of spermatogenesis were noted in these groups. Other effects in the treated groups included a decrease in the number of primary follicles and an increase in the degeneration of the follicular epithelium in the ovaries (except in the 0.2% and 0.8% groups); gastritis (except in the 0.6% and 0.8% groups); and degenerative myocardial fibres and focal infiltrations of leucocytes in the heart (except in the 0.2% and 1% groups).

It is not clear whether the absence of a dose–response relationship for the changes in ovaries, stomach and heart were related to the small number of animals used for histological examination (3/sex/group). A NOEL could not be set for this study (Salsbury Laboratories, 1962a).

In a study in rats fed dimetridazole in the diet at 0, 50 or 100 mg/kg bw/d for three months, no treatment-related effects were reported on clinical signs, body weight gain or urinalysis, but no data were provided for the study. No abnormalities were seen at histopathological examination but the examination was conducted in only a small number of animals (up to 2 animals/sex/group). A NOEL could not be determined for this study because of the deficiencies in the study (Hood, 1962a).

In a study conducted in a small number of dogs (1/sex/group), dimetridazole was given by gavage at 12.5 or 50 mg/kg bw/d for three months. Clinical signs included ‘blood-shot eyes’ with mydriasis and increased excitability in all dogs. One dog at 50 mg/kg bw/d had contracted muscles (abdominal and hind legs, rigor of the tail). According to the study author, clinical chemistry and urinalysis did not reveal any abnormality in liver or renal function. In the 50 mg/kg bw/d group, chronic pyelonephritis, fatty changes in the liver, hypocellularity in the bone marrow, and abnormal thyroid colloid were seen. No NOEL could be reliably set from this study due to lack of study details and the small number of animals employed (Hood, 1962a).

Dogs were given dimetridazole at 16, 33, 66 or 132 mg/kg bw/d po for 13 weeks. In the 132 mg/kg bw/d group, one dog died and the remaining dogs were killed *in extremis*. Bodyweight gain and food consumption were decreased in the treated groups. Ataxia, anorexia, convulsions and opisthotonos were seen at 66 and 132 mg/kg bw/d. It is not clear whether haematology, clinical chemistry, organ weights, gross pathology and histological examination were carried out. No NOEL was set from this study (Salsbury Laboratories 1962c; study was evaluated by JECFA, 1990).

Dogs (4/sex/group) were given dimetridazole at 0, 5, 10, 20, or 40 mg/kg bw/d po for 13 weeks. Except for one dog in the 40 mg/kg bw/d group which died while under anaesthesia for bone marrow biopsy, there were no deaths. There were no drug-related effects on clinical signs, bodyweight, ophthalmology, neurology, food consumption, urinalysis, haematology, biochemistry, organ weight or histopathology (Goyder *et al.* 1974; study was evaluated by the Australian Department of Health in 1986 and by JECFA, 1990). It appears that 40 mg/kg bw/d was the NOEL for this study.

2.2.4. Chronic studies

Female rats were given dimetridazole in the diet at doses of 0 or 0.2 % (approximately equivalent to 0 or 130 mg/kg bw/d) for 46 weeks, followed by a control diet containing no dimetridazole for 20 weeks (Bryan, 1970). Mortality was higher in the treated group. There were no treatment-related effects on bodyweight. At week 65, there was a biologically significant increase in the number of rats with benign mammary tumours (fibroadenomas) in the treated group. The incidence of multiple mammary tumours was also increased in the treated group. A NOEL could not be set for this study.

Diets containing 0, 100, 400 or 2,000 ppm were fed to groups of rats for 122 weeks (0, 3.8, 15 or 78 mg/kg bw/d in males; 0, 4.6, 18 or 94 mg/kg bw/d in females) (Lowe *et al.* 1976; study was evaluated by the Australian Department of Health in 1986; by JECFA in 1990). Mortality was increased in the 400 (females) and 2,000 (males and females) ppm groups. In the high dose group, bodyweight gains were 'slightly' lower in females, but food consumption was not affected by treatment. In this group, nodules appeared sooner and a higher incidence was noted when compared with the control and lower dose groups. In the 400 and 2,000 ppm groups, there was a dose-dependent increase in the incidence of benign tumours (adenoma, fibroadenoma, fibroma) of the mammary gland. An increase in tumour multiplicity was also observed in females of these groups. Malignant mammary tumours were not increased in any treated group. A NOEL of 100 ppm was set for this study.

Diets containing 0 or 10 ppm dimetridazole (0 or 0.45 mg/kg bw/d in males, 0 or 0.57 mg/kg bw/d in females) were fed to rats for 128 weeks (Lowe *et al.* 1977). At the end of the study, survival rates were decreased in treated males (survival was 32% and 12% in control and treated groups respectively). Treatment with dimetridazole had no effect on clinical signs, bodyweights or food consumption. In the treated group, increased relative liver and ovarian weights were seen in males and females respectively (without treatment-related histological changes). In the liver, increased incidences of congestion in males, and bile duct hyperplasia and parenchymal cell degeneration in females, were seen in the treated group. The number of rats with malignant tumours was slightly increased in the treated group. In the treated group, the incidence of pituitary adenomas was decreased in males while the incidence of mammary tumours (malignant) was slightly increased in females.

The biological relevance of the small increase in the tumour incidence was not clear and statistically not significant. Compared with other strains (SD and Wistar), the strain used in the study (CFY) was found to have a higher incidence (~2 fold) of spontaneous mammary tumours. A NOEL could not be set for this study (decreased survival and liver changes were observed at the only dose tested).

2.2.5. Reproductive toxicity study

Groups of weanling rats were maintained on a diet containing 0, 100 or 2,000 ppm dimetridazole (approximately 10 or 200 mg/kg bw/d) for ~80 days prior to the first mating and throughout the production of three generations. Dimetridazole markedly reduced weight gain and food intake of F_0 males in the 2,000 ppm dosing group. Except for increased mortality in 'F_{1b} offspring' (both treated groups), there were no other treatment-related effects. The mortality was attributed to the increased number of dams that ceased lactating (Dale 1975; study was evaluated by the Australian Department of Health in 1986; and by JECFA, 1990). A NOEL was not set.

In four studies in which diets containing 200, 500, 1,500 or 2,000 ppm dimetridazole were fed to sows and boars during the reproduction cycle, no treatment-related effects on reproductive traits and litter were observed (Anderson 1972, 1973, 1974; Hutchings and Evans, 1973). Similarly, diets containing 125 or 250 ppm dimetridazole had no effects on reproduction in turkeys over two generations. At 500 ppm, a drop in fertility in one generation was observed when turkeys were fed from one day old (Lucas *et al.*, 1967). PACC evaluated all five studies in 1986, but a NOEL was not set for each study.

2.2.6. Developmental toxicity study

Dimetridazole was administered by gavage to rabbits on gestation days 6 -18 at 0, 30, 60 or 120 mg/kg bw/d. Dose-related maternal toxicity, as evidenced by the reduction in food intake and bodyweight gain as well as abortions, was noted in all treated groups. Post-implantation loss was increased at the high dose. There was a slight reduction in foetal and placental weight (dose levels not stated). A NOEL could not be set for this study (Tesh *et al.*, 1988; study was evaluated by JECFA only, 1990).

2.3. Genotoxicity studies

2.3.1. Gene mutation in bacteria, fungi and cultured mammalian cells

In a few studies conducted in bacteria (mostly in *Salmonella typhimurium;* one study in *Escherichia coli*, *Citrobacter freundii* and *Klebsiella pneumoniae*), dimetridazole (≤282 µg/mL) in the presence and absence of metabolic activation was mutagenic (Voogd *et al.* 1974; Wang *et al.* 1975; Benazet & Cartier, 1977; Mourot, 1988). The mutagenic effect appeared to be dependent on bacterial nitroreductase activity since dimetridazole by itself (100 µg/mL), or its urinary metabolites collected from rats treated with dimetridazole (400 mg/kg bw po or iv), gave only negative results in mutagenicity tests conducted on a strain of *S. typhimurium* (TA 100 Frl) which did not have any nitroreductase activity (Thybaud *et al.* 1988).

In yeast (*Saccharomyces cerevisiae* D4), dimetridazole (500 µg/mL, without metabolic activation) was mutagenic (Voogd *et al.* 1980).

In mammalian cells (CHO-K1 cells), dimetridazole (≤7,500 µg/mL) did not induce forward mutation of the HGPRT gene in the presence or absence of metabolic activation. Severe cytotoxicity was seen at ≥5,000 µg/mL (cytotoxicity seems to be increased in the presence of metabolic activation) (Cordier & Bonneau, 1985).

2.3.2. Chromosomal effect assays *in vitro*

Dimetridazole was devoid of clastogenic activity when tested on CHO-K1 cell line at 500–2,800 µg/mL without metabolic activation and at 10–820 µg/mL with metabolic activation (Fournier & Cordier, 1986a).

2.3.3. DNA damage and repair *in vitro*

Dimetridazole did not induce DNA repair in Chinese hamster lung fibroblasts (200 µg/mL; Richold *et al.* 1981) and CHO cells (≤20 µg/mL, concentrations >20 µg/mL were cytotoxic; Ingham, 1981). However, positive results were observed in a comet assay in human lymphocytes (Re *et al.* 1997).

A comet assay is a technique that is used for detecting damage to the DNA of animal and human cells following exposure to test substances *in vivo* or *in vitro*. In conjunction with other genotoxicity assays and *in vivo* studies in animals, the comet assay provides information on the potential for a substance to cause DNA damage, which may lead to cancer.

In the comet assay, dimetridazole (71–354 µM for dimetridazole; 10–50 µg/mL) and metronidazole (58–292 µM) showed evidence of DNA damage as revealed by a significant and concentration-dependent increase in tail moment (comet length x quantity of DNA in the tail) when aerobic conditions were used in the assay. The DNA damage caused by these compounds was reduced under anaerobic conditions and was abolished in the presence of metabolic activation. Antioxidants (8-hydroxyquinoline, 52–258 µM; vitamin C, 285–2850 µM; catalase, 83–249 U/mL; and superoxide dismutase, 36–144 U/mL) induced a concentration-related protective response against the DNA damage caused by dimetridazole and metronidazole (Re *et al.* 1997).

2.3.4. Gene mutation *in vivo*

A dominant lethal mutation assay in mice gave negative results (dimetridazole at up to 1,000 mg/kg bw/d, route of administration not stated, Dale, 1975; Dale, 1977). In this assay, 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI at 75 and 750 mg/kg bw/d po), a metabolite of dimetridazole, also was negative (Dale, 1977).

In *Drosophila melanogaster* fruit flies, dimetridazole administered to adult males either in the diet at 0.7, 1.4 or 2.8 mM (toxic at 2.8 mM) or by 'injection' (0.2 μL) at 7.1 or 35 mM (toxic at 35 mM) did not show any evidence of sex-linked recessive lethality when treated males were mated with untreated females. When dimetridazole was fed to larvae at 0.7, 1.4, 2.8 or 4.4 mM (toxic at 2.8 and 4.4 mM), dimetridazole induced significant increases in lethal mutations in some experiments, but the results were not reproducible. Hence dimetridazole (as well as metronidazole) was concluded not to be mutagenic in this assay. Out of the nine 5-nitroimidazoles (including dimetridazole and metronidazole) tested in this assay, compounds such as ZK 26.173 and ZK 25.095 (moxnidazole) were 'clearly' mutagenic while nimorazole and ronidazole were 'marginally' mutagenic (Kramers, 1982).

2.3.5. Chromosomal effect assays *in vivo*

In two micronucleus assays in mice, dimetridazole administered at 305–915 mg/kg bw po or 220 mg/kg bw intraperitoneally did not significantly increase the incidence of micronucleated polychromatic red blood cells in the bone marrow (Oud *et al.* 1979; Fournier & Cordier, 1986b).

2.3.6. DNA damage and repair *in vivo*

In rats, dimetridazole at 1,000 mg/kg bw/d did not increase the incidence of unscheduled DNA synthesis in hepatocytes (Melcion & Cordier, 1988).

2.4. Hazard assessment

2.4.1. Adequacy of the database

The toxicological database on dimetridazole is poor in its coverage and quality. Many of the studies date back to the 1960s, and in some instances to the late 1950s. These studies are now of limited regulatory value because of the limited range of measurements made and/or the level of detail in the reports. Data gaps include:

- the availability of only JECFA evaluation summaries, and not full study reports for some pivotal studies including a carcinogenicity study in rats that was used previously to set the NOEL, reproductive and developmental toxicity studies (see Table 1)
- the absence of a carcinogenicity study in a second species
- no data on metabolites in mammals
- the absence of dermal toxicity, inhalational toxicity, and skin sensitisation studies conducted with the active constituent dimetridazole or its products.

These deficiencies are serious and there does not appear to have been any attempt to address them.

2.4.2. Metabolism and toxicokinetics

The metabolism and toxicokinetics of dimetridazole are not well characterised in laboratory animals or in humans, and hence the assessment of dimetridazole has to rely on data available for other 5-nitroimidazole chemicals and on data available in target species (studies in turkeys and pigs). Although a rat study was cited in a submission to PACC (February 1987) and to the JECFA (1990), no data have been submitted to OCS. It appears from the limited information provided that in rats, absorption (oral) and excretion of dimetridazole are ‘quick’. Dimetridazole is also reported to be 'extensively metabolised', with the formation (oxidation of the 2-methyl group) successively of 2-hydroxymethyl-1-methyl-5-nitro-imidazole (HMMNI) and 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA), which were excreted in the urine. Such metabolites were also found in turkeys and pigs. According to the JECFA (1990), metabolism in rats is similar to that in pigs. However, the OCS could not verify this.

2.4.3. Acute toxicity

Dimetridazole exhibits low oral acute toxicity. Clinical signs of toxicity in both rats and mice were sedation for 24 hours (Hood, 1962b). Haemorrhage in lungs and cervical lymph nodes was seen in rats when dimetridazole at very high concentrations (5,000, 10,000 and 20,000 mg/m^3) was administered by inhalation (for four hours).

Although data on the skin and eye irritation studies were not adequate since details about individual animal scores were not provided, there was no evidence of skin or eye irritation. There were no studies to determine either the acute dermal toxicity or potential for skin sensitisation.

Studies on the products were limited. Emtryl Soluble (containing 40% dimetridazole) showed low oral toxicity in mice and rats and was not a skin irritant. Only a skin irritation study was conducted in rabbits with Emtryl Premix (containing 22.45% dimetridazole) and this study did not reveal any significant skin irritation.

2.4.4. Short-term repeat-dose studies

Studies conducted in mice, rats and dogs gave only limited information. Deficiencies in these studies included limited parameters measured, insufficient numbers of animals in the tests, and/or the absence of a control group for comparison.

A mouse study monitored only one parameter, ie mortality (the maximum non-lethal dose was 500 mg/kg bw/d, po) and dimetridazole treatment was given only for five days. There was no control group in the study.

In a four-week study in rats given 50 or 100 mg/kg bw/d (presumably oral), there were no treatment-related effects reported on clinical signs, haematology, clinical chemistry or histology. However, histological examination was conducted on only 1 or 2 animals/sex/group and hence a meaningful interpretation of the study results is difficult.

In two dog studies, the number of animals used was low (2/group), and there was no control group in one study. From the available data, dogs appear to be more sensitive with respect to central nervous system effects than mice or rats, since ataxia was seen in dogs at ≥50 mg/kg bw/d po. It is not clear whether the central nervous system effect was a 'direct' (pharmacological or physiological) effect of dimetridazole or caused by haemorrhage in the spinal cord. Haemorrhages were seen not only in the spinal cord but also in different organs (kidneys, heart, spleen and liver, at ~270 mg/kg bw/d po). Toxic effects in kidneys (nephrosis), liver (central lobular cirrhosis in addition to haemorrhages) and lungs (proliferation of interstitial tissue) were also seen in dogs at ~270 mg/kg bw/d po while testicular toxicity (mild atrophy of seminiferous tubules, no mature spermatocytes, degeneration of spermatids) was seen at a lower dose (~90 mg/kg bw/d).

Because of study deficiencies and toxic effects seen at the low dose used in these studies, a NOEL could not be determined for any of these studies.

2.4.5. Subchronic studies

Two studies have been conducted in rats, but neither of these studies could be used for setting a NOEL in rats. One study was only in a summary form, and toxic effects (in testes, ovaries, stomach and heart) were seen at all tested doses. In a second rat study (evaluated in 1986; data not available for re-evaluation) there were no significant concerns except that the number of animals used for histological examination was low (1 or 2/sex/group).

Three studies were conducted in dogs, but only three of the five available studies were evaluated by the PACC in 1986. However, none of the study reports are now available for re-evaluating the data. Central nervous system effects, including ataxia and convulsions, were seen in two studies at doses ≥50 mg/kg bw/d. In a study evaluated in 1986, the NOEL was 40 mg/kg bw/d, the highest dose in the study.

Overall, in only one dog study could a NOEL (40 mg/kg bw/d) be set.

2.4.6. Carcinogenicity studies

Three studies have been conducted in rats to address the potential carcinogenicity of dimetridazole. However, a major deficiency in the database is that there was no carcinogenicity study in a second species ie mouse.

Only one dose was used in two of the studies and hence a dose-response relationship could not be assessed in these studies. A third rat study used three doses (100, 400 and 2,000 ppm) and this study was evaluated by the Australian Department of Health in 1986. In this study, a NOEL of 100 ppm (3.8 mg/kg bw/d) was set, based on benign mammary tumors occurring at higher doses. However, the United States Food and Drug Administration (US FDA) did not accept this NOEL since 40%, 54%, 90 % and 56% of males and 22%, 32%, 14% and 14% of females in the 0, 100, 400 and 2,000 ppm groups respectively were not subjected to histological examination to detect the presence of mammary tumors.

Another study conducted with a lower dose of dimetridazole (10 ppm) showed that there was no statistically significant increase in mammary tumors, but a problem in this study as well as in the previous study (in which three doses were used) was that the rat strain used (CFY) had a high background incidence of mammary tumors in females (~70% of animals had benign or malignant mammary tumors; Lowe *et al.* 1977). Because of the high background incidence of mammary tumors in this strain (when compared to the incidences of 25% in SD and 36% in Wistar rat strains) it is unlikely that small treatment-related increases in mammary tumor incidences would be detected in this strain.

A study was conducted in SD rats but only one dose was used (0.2% in diet, 2,000 ppm). In this study, benign mammary tumors were increased significantly at the tested dose but the effects of lower doses in this strain are not known. Another drawback of the SD rat study was that only females were tested and hence carcinogenic effects, if any, in SD males are not known. However, in the CFY strain, incidences of mammary tumors were increased at both 400 and 2,000 ppm in males and hence mammary tumors in this strain of rats are not sex-specific.

Other than mammary tumors, there were no biologically significant increases in the incidences of other tumors in any of the three carcinogenicity studies conducted in rats.

Dimetridazole is a 5-nitroimidazole and several related 5-nitroimidazoles are known to be carcinogenic. Thus, the 5-nitroimidazoles – metronidazole, ipronidazole and ronidazole –have been reported to be carcinogenic (see Table 1 below). A common finding with all three compounds was that the compounds increased mammary tumors in rats (similar to dimetridazole) and lung tumors in mice. Based on structural similarities, dimetridazole may also increase lung tumors in mice, although this potential has not been tested. No tumors of any kind have been reported in humans treated with metronidazole. Metronidazole is normally used therapeutically for less than a month, although it may be used for up to three months in cases of Crohn's disease. However, humans are not usually exposed to metronidazole for a prolonged period. With respect to dimetridazole, prolonged human exposure is possible by eating meat containing dimetridazole and metabolite residues. While metronidazole has been taken during all stages of pregnancy with no apparent adverse effects, its use during the first trimester is not recommended.

Table 1. Comparative toxicity of some 5-nitroimidazoles

Chemical	Genotoxicity	Carcinogenicity	Reproductive and developmental toxicity
Metronidazole[1]	Genotoxic *in vitro* and *in vivo*[1]. The Ames test was positive while several nonbacterial tests in animals were negative. In patients with Crohn's disease, metronidazole increased chromosomal abnormalities in circulating lymphocytes[2].	Carcinogenic in mice (≥2 mg/kg bw/d, gavage) and rats (≥35 mg/kg bw/d, gavage). Mammary tumours in rats; lymphomas and lung adenomas in mice[1].	Reproductive toxicity in long-term studies in mice and rats: hypo-spermatogenesis; decreased prostate and testis weights[1]. In mice, increased incidences of dead and malformed foetuses at 15 mg/kg bw/d ip[1]. EMEA concluded that 'teratogenicity has not been sufficiently tested'[1].
Tinidazole	Mutagenic in the Ames test[2].	Animal carcinogenic studies are inadequate to exclude tumorigenic potential[2].	Animal studies suggest that tinidazole may have teratogenic potential[2].
Ipronidazole	Mutagenic in bacteria; inadequate study design in mammalian test systems[3].	Increase in lung tumours in mice at 1,000 ppm (150 mg/kg bw/d), and in mammary tumours in rats at 2,000 ppm (110 mg/kg bw/d)[3].	In a 3-generation study in rats, reduced growth in pups and dams at 2,000 ppm (100 mg/kg bw/d). No embryo-foetal toxicity in mice and rats at ≤10 and ≤100 mg/kg bw/d respectively[3].
Ronidazole	Genotoxic in bacteria and in the sex-linked recessive lethal test in *Drosophila melanogaster*; weakly positive or negative in the bone-marrow cytogenetic assay in mice; negative in the micronucleus tests and a dominant lethal assay[3].	Lung adenoma or carcinoma in mice at 20 mg/kg bw/d; benign mammary tumours in rats at 10 and 20 mg/kg bw/d[3].	Testicular toxicity in chronic studies[3]. In a 3-generation study in rats, no adverse effects on reproduction except for decreased number of pups at 800 mg/kg bw/d. In two other studies in rats, decreased foetal weight at 100 mg/kg bw/d[3]. In mice and rabbits, at up to 200 and 30 mg/kg bw/d respectively, maternal toxicity at the high dose, but no significant teratogenicity[3].

[1]European Agency for the Evaluation of Medicinal Products (EMEA), July 1997
[2]MIMS, 2003
[3]JECFA, 1990.

The mechanism of dimetridazole-induced mammary tumors is not clear. As with other 5-nitroimidazoles, and as seen in target animal residues studies, dimetridazole undergoes (or is expected to undergo) oxidation at the 2-methyl group, leading to the formation of metabolites such as HMMNI, MNICA and their conjugates, reduction (metabolites such as nitroso- and hydroxylamino-compounds) and ring scission (acetamide is a possible metabolite). While acetamide is a carcinogen in rats, compounds with a nitro group (dimetridazole and some of its metabolites including HMMNI) have the potential to initiate neoplastic processes as a result of covalent binding to DNA (US FDA, 1986).

In the carcinogenicity studies, the relevance of the rat as a model to address the carcinogenicity potential of dimetridazole and its metabolites is not clear. This is because data on the metabolite profile (including quantitative data) of dimetridazole in rats have not been provided, and residues in target species have not been fully characterised, and hence it is not known whether the metabolites formed in rats are qualitatively and quantitatively similar to the residues formed in target animals.

Overall, there are significant data gaps in addressing the carcinogenic potential of dimetridazole. On the basis of available data, dimetridazole is clearly capable of inducing tumors (benign lung neoplasms) in rats, and judging from the effects of other 5-nitroimidazoles in mice, dimetridazole may also be carcinogenic in mice. Dimetridazole and several 5-nitroimidazoles are mutagenic in bacteria, and with new evidence (comet assay) showing that dimetridazole, like metronidazole, can cause DNA damage in mammalian cells, the main concern is that dimetridazole has the potential to be a 'genotoxic carcinogen'.

2.4.7. Genotoxicity studies

Dimetridazole is clearly mutagenic in a variety of *in vitro* bacterial assays including *Salmonella typhimurium* (TA100, TA100-FR, TA1530, His G46, TA1531, TA1532, TA1534 (Lindmark & Muller, 1976; Rosenkranz *et al.* 1976), *Klebsiella pneumoniae* (Voogd *et al.* 1979, 1974), *Escherichia coli* (Voogd *et al.* 1974) and *Citrobacter freundii* (Voogd *et al.* 1974; Voogd *et al.* 1979). Some of the studies were not assessed by the Australian Department of Health since a few full study reports which were submitted to JECFA for evaluation were not submitted to that Department.

The Australian Department of Health's evaluation reports in 1986 and 1987 did not contain any statement regarding mutagenic activity of dimetridazole in bacteria and it appears that no study reports on bacterial mutagenic activity were submitted to the Department in these years. Later, the Department noted that an Ames study in *S. typhimurium* (Mourot, 1988) was evaluated by JECFA in 1990, but in spite of a request (February 1991) by the PACC to the sponsor (Rhone-Poulenc) to submit this study for evaluation, the study was never submitted. The mutagenic effect of dimetridazole in bacteria has been claimed to be due to the presence of nitroreductase enzyme in the bacterial cells, but no data have been submitted to the OCS in support of this claim.

A concern is that structural analogues of dimetridazole, such as ipronidazole, ronidazole, metronidazole, 2-methyl-5-nitroimidazole, ornidazole and nimorazole, have also been shown to be mutagenic (see Table 1 above). According to the US FDA (1986), at least twenty 5-nitroimdazole chemicals have been found to be mutagenic to *S. typhimurium*. Metronidazole and ipronidazole are mutagenic in the same bacterial

systems as dimetridazole, namely *S. typhimurium, E. coli, K. pneumoniae* and *C. freundii* (Mohn *et al.* 1979; Voogd *et al.* 1977, 1979; Voogd, 1981). The fact that all three compounds are mutagenic in the same test systems is especially noteworthy because ipronidazole and metronidazole have been shown to be mutagenic in mice (*The regulation of animal drugs by the Food and Drug Administration* 1985; Cavaliers *et al*., 1983, 1984; Rustia & Shubik, 1979). Thus 5-nitroimidazoles, as a group, have the potential to induce mutagenic effects in bacteria.

Although dimetridazole is mutagenic in bacteria and also in some eukaryotic cells (*Saccharomyces cerevisiae*), other tests conducted in mammalian cells (*in vitro*) and in insects (*D. melanogaster)* and mammals (mice, rats) indicated that dimetridazole was not genotoxic in these assay systems. The positive effect seen in bacteria and in *S. cerevisiae* was attributed to the presence of nitroreductase activity in these cells, since dimetridazole did not show any mutagenic activity in a bacterial strain deficient in this enzyme (*S. typhimurium* TA 100 Frl; Thybaud *et al.* 1988). It is to be noted that this study (Thybaud *et al.* 1988), as in the case of the Ames test (Mourot 1988), was evaluated by JECFA but not independently assessed by the Australian Department of Health. Moreover, two other studies in mammalian cells (both evaluated by JECFA, 1990; unscheduled DNA synthesis in Chinese hamster lung fibroblasts, Richold *et al.* 1981; *in vivo* study in rats on DNA damage and repair, Melcion & Cordier, 1988) were not submitted for evaluation in spite of a request (in 1991) by the PACC to the sponsor.

Most of the *in vitro* assays were conducted in the presence and absence of metabolic activation with S9 prepared from rat liver but it is not clear whether the residues found in target animals (metabolites such as HMMNI and MNICA, or their conjugates) were also formed in the genotoxic assays, in the presence of metabolic activation by S9. A metabolism study has been reported as having been conducted in rats (as per a submission to the PACC in February 1987; RMB Animal Health Ltd, May & Baker report RES/2498) but the study data do not appear to have ever been submitted. In this rat study, the metabolism was reported to be similar to that in turkeys and pigs, but this information could not be verified because of the absence of data. Hence it is not clear whether the *in vitro* (with metabolic activation) and *in vivo* models used are relevant to address the genotoxic potential of dimetridazole metabolites.

The genotoxicity study by Thybaud *et al.* (1988; data not submitted) appears to have addressed this issue at least partly since the study report (as evident from the study title) appears to discuss (or provide evidence for) the roles of liver S9 mix, bacterial nitroreductases and *in vitro* metabolism in the liver and the intestinal flora on the mutagenic activity of dimetridazole in the Ames test.

The metabolites HMMNI and MNICA (formed from HMMNI) are also suspected to be mutagenic. This potential was not investigated in bacterial assay systems which showed positive responses to the parent compound. In a dominant lethal mutation assay in mice, both dimetridazole and a metabolite 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) gave negative results. However, other metabolites of dimetridazole were not tested in this assay. Although adequate studies are not available to address the mutagenic potential, it is known that an analogous 2-hydroxymethyl metabolite of metronidazole is five to 10 times more mutagenic than the parent compound in *S. typhimurium* TA 1535 (US FDA, 1986). Hence, the mutagenic potential of the metabolites of dimetridazole cannot be ruled out.

The results of a comet assay conducted in 1997 (Re *et al.* 1997) in human lymphocytes are of concern with respect to chromosomal damage. In this *in vitro* study, dimetridazole and metronidazole, in the absence of metabolic activation by S9, were found to cause significant and concentration-dependent DNA damage. Metabolic activation reduced the genotoxic damage and hence it is likely that dimetridazole *per se* (or its intracellular metabolites) is capable of causing genotoxicity. The magnitude of the genotoxic response was reduced by anaerobic conditions and abolished by antioxidants (8-hydroxyquinoline, vitamin C, catalase and superoxide dismutase). These results suggest that dimetridazole induces oxidative DNA damage, a mechanism that may be different to that operating under anaerobic conditions. Hence, dimetridazole may have the potential to induce genotoxicity in human cells, functioning in an aerobic environment.

Although dimetridazole did not show any effect in chromosomal effect assays (*in vitro* or *in vivo*), increases in the incidences of chromosomal aberrations have been reported in metronidazole-treated mammalian cells under aerobic conditions (Korbelik & Horvat, 1980). Some investigators have suggested that the genotoxicity of metronidazole may have been at least partly associated with the reduction of the nitro group of metronidazole by nitroreductase under anaerobic conditions (Korbelik & Horvat, 1980; Rosenkranz *et al.* 1982; Rosenkranz & Mermelstein, 1983). A similar mechanism may be responsible for the mutagenic effects of dimetridazole in bacteria. However, in the comet assay (Re *et al.* 1997), the magnitude of the genotoxic response was reduced by anaerobic conditions and abolished by the inclusion of antioxidants (8-hydroxyquinoline, vitamin C, catalase or superoxide dismutase). These results suggest that dimetridazole induces oxidative DNA damage, a mechanism that may be different from that operating under anaerobic conditions, thus suggesting dimetridazole may have the potential to induce genotoxicity in aerobic human cells. For these reasons, mutagenic effects of dimetridazole observed in bacterial assays cannot be dismissed as bacterial-specific effects which have no relevance to humans.

The reason for the absence of genotoxic effects in the *in vivo* studies is not clear. However, as pointed out before, until the metabolism of dimetridazole in the species used (*D. melanogaster*, mice and rats) is better characterised, the results of the *in vivo* studies (not only genotoxicity studies, but also other toxicity studies) should be interpreted with some caution.

The methodology used in an *in vivo* DNA damage and repair study (only one study; Melcion & Cordier, 1988) has been questioned. The EU's Scientific Committee on Animal Nutrition had noted that this study had not been performed to current OECD guideline 486 that was adopted in 1997. This guideline recommends an early (at two–four hours post-dose) as well as a later sampling time point (12–16 hours), noting that some compounds such as dimethylnitrosamine would not have been detected as positive if only the later sampling time point was used. In the Melcion & Cordier study, the only sampling time was 15 hours. It is to be noted that this study was requested by PACC (1991), but the study was not submitted by the sponsor. Based on available data, the Scientific Committee on Animal Nutrition concluded that further data were needed to provide adequate assurance that the mutagenic activity seen *in vitro* was not expressed *in vivo*.

Overall, there are data deficiencies in trying to assess the genotoxic potential of dimetridazole. The available information indicates that the potential of dimetridazole to be genotoxic cannot be ruled out.

2.4.8. Reproductive and developmental toxicity

In the literature, metronidazole, ipronidazole and ronidazole have been reported to adversely affect reproductive performance and developmental toxicity (see Table 1). In chronic studies, metronidazole and ronidazole have been previously shown to cause testicular toxicity.

Studies on dimetridazole revealed testicular toxicity (histological changes) at high doses (≥200 mg/kg bw/d) in subchronic studies in rats. However, male fertility was not affected in a three-generation reproduction study in rats and hence testicular effects are unlikely to be a significant concern. In this three-generation reproduction study, increased pup mortality was seen with dimetridazole treatment in only one generation (F_{1b} offspring), an effect which appeared to be due to decreased lactation in dams. It is not clear whether dimetridazole has a direct effect on lactation or whether lactation was affected by some other mechanism eg the presence of mammary tumours. Since only one generation was affected, it was not completely clear whether the changes seen were treatment-related. Because of the uncertainties, a NOEL could not be set for this study.

The potential for developmental effects was only partially investigated. Only one species (rabbits) was used to study the effect of dimetridazole on developmental toxicity but the study (Tesh *et al.* 1988) was never submitted to the Australian Department of Health. In the evaluation by JECFA (dimetridazole at 30, 60, 120 mg/kg bw/d po), post-implantation loss (120 mg/kg bw/d) was increased. A 'slight' reduction in foetal and placental weights (dose levels not stated) was seen in the treated groups, but the effects were associated with maternal toxicity (dose-dependent reduction in food intake and bodyweight gain; abortion) in all treated groups. A NOEL was not established for maternal toxicity, and in the absence of detailed study results, a NOEL could not be set for developmental effects either.

2.4.9. Concerns evident in toxicity studies

The following concerns were evident in the toxicity studies and in the database submission:

- crucial studies submitted to overseas agencies were not submitted in Australia
- there is new evidence that dimetridazole is genotoxic in mammalian cells (comet assay); hence there is a potential that dimetridazole could be a genotoxic carcinogen
- data on metabolism and toxicokinetics of dimetridazole in turkeys and pigs are referenced in this review but no data on metabolism and toxicokinetics of dimetridazole in laboratory mammals were submitted to the review. Such data are crucial to understand the relevance of animal models used in toxicity studies in determining the human toxicity of residues formed in target animals
- there are no data in a second species for carcinogenicity
- data in a second species for developmental toxicity were not available to OCS for assessment although data for dimetridazole from pig studies were considered in 1986 by PACC

- NOELs could not be set in many studies because of deficiencies in the studies; the previously used NOEL of 3.8 mg/kg bw/d in a rat carcinogenicity study could not be reliably considered as the overall NOEL to set an ADI because of the above concerns.

2.5. Dose levels relevant for risk assessment

The OCS used a two-year dietary study in rats (Lowe *et al.*, 1976) to set the previous NOEL in 1986. In this study, the NOEL was found to be 3.8 mg/kg bw/d based on benign mammary tumours (Lowest Observed Effect Level (LOEL): 15.1 mg/kg bw/d). The maximum safety factor of 2,000 was used to derive an ADI of 0.002 mg/kg bw/d since the database was incomplete.

In the previous evaluation, a NOEL of 40 mg/kg bw/d was established in a 13-week oral dog study (see Table 2 below). A NOEL could not be set for many other studies. The OCS toxicological evaluation of dimetridazole has found the available database insufficient to determine a reliable overall NOEL. Hence, the OCS could not determine an overall NOEL and ADI nor affirm the previous ADI that was set in 1986. Furthermore, the available data are insufficient to set an ARfD.

Table 2. Establishment of a NOEL

Species	Study type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
Rat	2-year dietary	3.8	15.1	Benign mammary tumours	Lowe *et al.*, 1976
Dog	13-week oral	40	40 mg/kg bw/d was the highest dose used.	No drug-related effect	Goyder *et al.* 1974

An overall NOEL could not be determined because of data deficiencies.

2.5.1. Diet

Australian Total Diet Survey and Australian National Residue Survey

The 1999–2,000 Australian National Residue Survey, conducted under the auspices of the then Australian Government Department of Agriculture, Fisheries and Forestry monitored dimetridazole residues in pigs but not poultry. No residues were found in pig muscle tissue (limit of reporting 0.001 mg/kg) from 148 analyses. Similar results were noted in the 1997 Australian National Residue Survey. Dimetridazole has not been included in the current or previous Australian Total Diet Surveys (formerly Market Basket Surveys).

Acute and chronic dietary intake

Acute and chronic dietary intake calculations are generally performed by the APVMA and Food Standards Australia New Zealand.

2.5.2. Water

Based on its registered veterinary uses, dimetridazole residues should not be found in water or water catchment areas. Therefore, no health level for dimetridazole in drinking water was set.

2.5.3. Non-dietary exposure considerations

This consideration was not evaluated in the toxicology assessment. Refer to Section 4, Occupational Health and Safety Assessment.

2.6. Consideration of public health standards

2.6.1. Approval status

A list of registered products and their approved uses is presented in Appendix B.

2.6.2. NOEL, ADI and ARfD

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health. It is calculated by dividing the overall NOEL from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans, intra-species variation, the completeness of the toxicological database, and the nature of the potential toxicologically significant effects.

The former ADI for dimetridazole, set by the OCS in 1986, was 0.002 mg/kg bw/d. It was based on a NOEL of 3.8 mg/kg bw/d from a two-year chronic dietary study in rats and the incidence of benign mammary tumours at the next highest dose using a 2,000-fold safety factor due to poor data supporting registration. The OCS' evaluation indicated that there are significant data gaps, and new concerns (regarding genotoxic potential) and hence a NOEL or ADI/ARfD could not be set using current available data. Therefore, the OCS could not confirm the previously established NOEL or ADI.

2.6.3. Poison scheduling

Dimetridazole is currently listed under Schedule 4 (Prescription Animal Remedy) of the SUSDP.

2.6.4. First aid instructions and safety directions

Since the use of dimetridazole in companion animals and birds is supported from a toxicological perspective, the current entry in the Handbook of First Aid Instructions and Safety Directions (FAISD Handbook), 'If poisoning occurs, contact a doctor or poisons information centre', should be retained.

At present, there are two products approved for use in companion animals and birds: Emtryl Soluble Dimetridazole Soluble Powder (containing 40% dimetridazole, w/w) and CCD Dimetridazole (Water Soluble Powder) (containing 100% dimetridazole, w/w). Currently there are no safety directions for dimetridazole products. No safety directions are required for the product CCD Dimetridazole (Water Soluble Powder) which contains only the active constituent. However, Emtryl Soluble Dimetridazole Soluble Powder contains calcium lignosulfonate, which is reported to cause contact allergy at or above 2%. Hence, the following statements are recommended for the product label:

Safety directions

Dimetridazole containing calcium lignosulfonate	
Repeated exposure may cause allergic disorders	180
Wash hands after use	351

3. IMPLICATIONS OF THE TOXICOLOGY ASSESSMENT

3.1. Uses of dimetridazole

As a result of the toxicological database not containing sufficient data to support the previous ADI, the OCS deleted this entry from the Australian ADI List published at http://www.tga.health.gov.au/docs/html/adi.htm. Consequently, uses of dimetridazole in food-producing species were not supported.

Because of the absence of alternative therapeutic agents, the APVMA initially proposed to develop a regulatory approach that would have allowed the continued use of dimetridazole in pigeons, other caged birds and game birds not intended for human consumption, but would have restricted the use in poultry to broiler breeders and turkey breeders. This initial approach needed to be supported from a residues perspective.

3.2. Residues

With respect to residues, this regulatory approach would have required a number of amendments to use practices and the MRL Standard, including:

- changes to the existing MRL entries for dimetridazole in pig and poultry meat and offal as well as the establishment of an MRL Standard for eggs
- establishment of suitable withholding periods so that there should be no residues of dimetridazole in food intended for human consumption
- changes to the residue definition of dimetridazole to account for metabolites which could have the same toxicological concerns as the parent compound.

3.3. Trade

With respect to trade, there should have been no trade implications associated with the above-mentioned regulatory approach since commodities exported from Australia should be free of residues of dimetridazole.

3.4. Occupational health and safety (OHS)

From an OHS perspective, if the use of dimetridazole were restricted to non-food-producing species as a regulatory outcome, the risk to users and workers from exposure to dimetridazole should be assessed and mitigated.

The Australian Material Safety Data Sheet for dimetridazole should be updated to include information consistent with the findings of this review.

These implications have led to subsequent OHS and residues assessments, which are discussed in Sections 4 and 5.

4. OCCUPATIONAL HEALTH AND SAFETY (OHS) ASSESSMENT

While the review focused primarily on toxicology issues, some of the findings are relevant to OHS. In reviewing the available information, it was recognised that data were not available to enable the identified concerns for worker safety to be comprehensively assessed. There were no exposure data available to estimate exposures, or suitable default methods available to assess risk to workers or domestic users. Therefore, a qualitative risk assessment was undertaken rather than a full OHS risk assessment that is based on margins of exposure. The OHS section of OCS conducted this assessment.

4.1. Hazard overview

4.1.1. Toxicity and hazard classification of dimetridazole and its products

From the toxicology assessment of available acute studies, the OHS assessment noted the toxicity profile of dimetridazole and the unavailability of data relating to the acute toxicity of dimetridazole or its products by the dermal or inhalation route, and the potential for skin sensitisation.

The OHS assessment also noted the difference in toxicity between products containing 40% or 22.5% w/w dimetridazole. One registered product contains calcium lignosulfonate (CAS No. 8061-52-7) at a concentration that is likely to cause contact allergy.

Based on the median oral LD_{50} from acute toxicity studies in mice and rats, and its genotoxic potential, dimetridazole is classified as being hazardous. Therefore, the following risk phrases (and cut-off concentrations) apply in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2002 draft).

R22 Harmful if swallowed

R68 Possible risk of irreversible effects (Mutagen category 3)

Concentration cut-off:	greater than or equal to 25%	R68, R22
	greater than or equal to 1% but less than 25%	R68

All six products are also classified as hazardous because the concentration of dimetridazole in the registered products ranges from 20% to 100%.

4.2. Use profile and occupational exposure assessment

Two use scenarios have been identified for the six registered products. In the first scenario end users add the product to feed in mixers, or to the drinking water, at the same time that they incorporate vitamin and mineral premixes.

In the second scenario, feed millers prepare medicated feed in feed mills where mixing occurs. Details of these operations are not available. The use profile of each registered product is listed in Table 3 below.

Table 3. Use profiles for dimetridazole products

Product name and pack size	DMZ* concentration (percentage of DMZ)	Species indicated	Label instruction (maximum and minimum % of DMZ)
CCD DMZ 225 Premix (Dimetridazole) (20 kg)	225 g/kg (22%)	Turkeys Chickens Pigs	For treatment: 2.2 kg/tonne feed for 7–14 days (0.06% maximum) For prevention: 340 g/tonne feed, fed continuously (0.01% minimum)
CCD Dimetridazole (Water Soluble Powder) (25 kg)	1,000 g/kg (100%)	Turkeys Pigs Caged birds	For prevention (turkeys and pigs): 120 g/1,000L of water (0.01% minimum) For prevention (caged birds): 250–600 mg/L water (0.06% maximum)
Dimetridazole FG (20 kg)	200 g/kg (20%)	Pigs	1,000 g/tonne of finished feed (0.02%)
Emtryl Premix for Feed Medication (20 kg)	200 g/kg (20%)	Pigs Poultry	2.5 kg of the product per tonne of finished feed (0.05% maximum) 375 g/tonne (0.01% minimum)
Emtryl Soluble Dimetridazole Soluble Powder (25 kg)	400 g/kg (40%)	Pigs Turkeys Chickens Pigeons	25 g/40 L water (0.02% maximum) 25 g/120L water (0.01% minimum)
Bronson and Jacobs Dimetridazole (DMZ) Oral (25 kg)	1,000 g/kg (100%)	Turkeys Pigs	500 g/tonne finished feed (0.05% maximum) 75 g/tonne finished feed (0.01% minimum)

*DMZ = dimetridazole

Workers who are considered at potential risk of exposure by inhalation and/or dermal contact have been identified and grouped as those who are:

- involved in the manufacture/formulation (suppliers) of dimetridazole products
- transporting the products
- preparing the medicated feed in feed mills
- using the products.

Persons at greatest risk of exposure are likely to be those involved in feed milling processes, where dermal and inhalation (dust) exposure to dimetridazole products is possible. Workers who are exposed to mixes are unlikely to be significantly exposed to dimetridazole due to low concentrations of 0.01% to 0.06% of dimetridazole in the mixes. End users may also come into dermal contact with dimetridazole during preparation of the final mix.

Now that the ADI was withdrawn and the registered products are no longer used in food-producing animals, it was not possible to estimate the quantity of dimetridazole that will be used for the remaining label uses. There is a possibility that with this limitation on use, manufacture/formulation of dimetridazole products in Australia will be significantly reduced; therefore, feed-milling operations may no longer be required.

The OHS assessment has determined that Good Manufacturing Practices and Hazard and Operability Studies could adequately control worker exposure during manufacture and formulation.

4.3. Occupational risk assessment

This assessment is qualitative in nature since it is not based on margins of exposure.

4.3.1. Risks associated with manufacture/formulation and transportation of dimetridazole products

Of the six registered dimetridazole products, five are manufactured in Australia. It is generally accepted that APVMA-registered manufacturers work under conditions of Good Manufacturing Practice, which includes attention to OHS by means of plant Hazard and Operability Studies. Several 5-nitromidazole analogues, which possess similar toxicological profile, are manufactured in Australia without reports of adverse effects such as increased incidence of cancer in workers involved in manufacturing. Risks during manufacture are therefore likely to be low.

Risks during transportation of dimetridazole products will be minimal as all the products are sealed in bags. Packaging requirements are regulated under the Department of Transport's Australian Code for the Transport of Dangerous Goods by Road and Rail, which also addresses appropriate emergency procedures when packaging is breached.

4.3.2. Risks associated with exposure during end use including workers preparing the medicated feed in feed mills

Data on dermal and inhalation toxicity, and eye and skin irritant studies in animals, are not available to characterise risks associated with the use of dimetridazole products. Risks will be highest during preparation of the feed in feed mills and on farms. During on-farm handling and mixing of products containing 20–100% dimetridazole, end users may be exposed via airborne dust. Once the feed is prepared for use, risks are likely to be minimal as the prepared feed will contain 0.01 to 0.06% of dimetridazole which is below the NOHSC concentration cut-off value of 1% for occupational genotoxicity hazard. Risks will be further reduced by any reduction in quantities handled, as a result of decreased usage.

4.4. OHS review outcomes

Exposure data would be required in order to characterise any risk to workers potentially exposed to dimetridazole and dimetridazole products. However, it is recognised that since the quantities of dimetridazole formulated and used in Australia are likely to be dramatically reduced as a consequence of the ADI withdrawal, it is unlikely that such data will become available.

Due to the lack of available worker exposure data and uncertainties over the NOEL for dimetridazole, the OHS assessment has attempted to identify exposure control/mitigation measures based on the available information. Safety directions and additional control measures that will mitigate potential acute and long-term occupational risks have been identified now that the APVMA has decided to support the continued registration of dimetridazole products for non-food-producing animals such as caged birds and pigeons.

4.5. Conclusions

The conclusions in the OHS assessment address:

- the requirement for further information to characterise risks to workers
- evaluation of label requirements
- suggested formulation change
- the requirement for additional OHS control measures under the NOHSC National Model Regulations for the Control of Workplace Hazardous Substances.

4.5.1. Requirement for further information

The OHS risk mitigation measures, which the qualitative risk assessment has identified, are relevant to the use of dimetridazole in pigeons, game birds and caged birds. However, if in future registrants, for example, were to apply to extend this current use pattern to a different host species or to change the dosage form from a water-based to an in-feed medication, OCS may require additional information to conduct a more detailed OHS exposure assessment. Such information may include, but is not limited to: details on the manufacture, formulation, milling and end use of the product; a projection of the quantity of product to be supplied; number of workers involved in manufacturing and formulation of the product; number of feed millers; and worker exposure data if available. This information would help to better characterise the risks to workers.

4.5.2. Labelling requirements

A number of inadequacies have been identified on current labels for dimetridazole products with respect to instructions relating to OHS. As a result, label recommendations are made to reduce current acute and chronic OHS risks.

Warning statement

The following warning should be included on all approved labels for dimetridazole:

> Dimetridazole is a possible genotoxic chemical.

Safety directions

In addition, all approved products are required to include the following safety directions:

> When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge).
>
> After each day's use wash contaminated clothing, gloves and respirator and if rubber wash with detergent and warm water.

These safety directions are considered sufficient to significantly reduce potential risks from both acute and repeated exposure to dimetridazole products.

Reference to Material Safety Data Sheet

Each approved label for dimetridazole should refer to a Material Safety Data Sheet for additional information.

4.5.3. Formulation change

The main concern for workers has been identified as potential inhalation of dust that contains dimetridazole from mixing and milling operations. In this regard OCS (OHS) considers that a change in formulation to reduce potential exposure to product dusts would significantly reduce risks to workers.

Without taking into account issues of feasibility, OCS (OHS) recommends that product suppliers consider reformulation of the products into water-soluble sachets. OCS (OHS) notes that two products intended for use in caged birds and pigeons are currently administered in drinking water and therefore this should not be an issue with regard to treatment efficacy.

Should products be reformulated so that potential inhalation of product powder/dust becomes negligible during normal work practices, the requirement for wearing a respirator when opening the container or preparing the mix could be cancelled.

4.5.4. Additional control measures under NOHSC National Model Regulations for Control of Workplace Hazardous Substances (NOHSC 1994a)

The following OHS control measures outlined in paragraphs 4.5.4.1 and 4.5.4.2 are integral to the safe use of dimetridazole. Compliance with labelling instructions alone may not provide sufficient risk mitigation. These additional recommendations are made under the NOHSC National Model Regulations for Control of Workplace Hazardous Substances under which all pesticide manufacturers and users should operate. The intention is for the relevant agencies in all states and territories to adopt and enforce these recommendations. Product registrants, users and OHS agencies are expected to be aware of these additional risk mitigation measures.

Availability of labels for dimetridazole

Labels prepared in accordance with NOHSC National Code of Practice for the Labelling of Workplace Substances (NOHSC 1994b) should be available to all workers involved in the manufacture or formulation of dimetridazole products. The labels should state the hazard classification for dimetridazole, including its genotoxicity potential.

Material Safety Data Sheets for dimetridazole products

Material Safety Data Sheets should be prepared in accordance with NOHSC National Code of Practice for the Preparation of Material Safety Data Sheets (NOHSC 1994c). A material safety data sheet should be available to all users of the products. It should reflect the hazard classification outlined in paragraph 4.1.1 and potential genotoxicity of dimetridazole products. Personal protective equipment recommended for handling all dimetridazole products should include a chemical resistant overall buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator with dust cartridge.

5. RESIDUES ASSESSMENT

5.1. Introduction

This assessment was based on existing available residues data for poultry as no new residues data were provided. In conducting this assessment, the APVMA's Chemistry and Residues Program took into account the fact that excess fertilised eggs and meat from culled replacement pullets/breeders and spent hens may be sold or processed for human consumption. The Chemistry and Residues Program further noted the OCS recommendation that the use of dimetridazole in food-producing animals be discontinued.

In the absence of an ADI and/or an ARfD for dimetridazole, no residues of dimetridazole would be acceptable in edible commodities. Therefore, the residues assessment was initially conducted to determine whether available residues data could support a regulatory approach permitting the continued use of dimetridazole in squab pigeons and game birds, but restricting the use in poultry to breeders. Chicken layer hens and broilers are excluded from treatment.

5.2. Toxicological information

The APVMA's Chemistry and Residues Program considered OCS' findings that there are insufficient toxicology data to set an ADI or ARfD, and noted its decision to withdraw the ADI for dimetridazole which had been established in 1986. Existing health standards for dimetridazole are provided in paragraphs 2.5 and 2.6.2 in this report.

5.3. Current dimetridazole approved use patterns in poultry

Of the five registered products containing dimetridazole that are approved for use in poultry, the pioneer products Emtryl Premix for Feed Medication (P38037) and Emtryl Soluble Dimetridazole Soluble Powder (P38038) were first considered for registration in 1986. Residues data were submitted with the applications. The remaining three products – Bronson and Jacobs Dimetridazole (DMZ) Oral (P50141), CCD Dimetridazole (Water Soluble Powder) (P50743) and CCD DMZ 225 Premix (Dimetridazole) (P52812) – are generic products, and no additional residues data were provided with the applications for registration.

The currently approved use patterns for dimetridazole in poultry are listed in Table 4.

Table 4. Currently-approved use patterns for dimetridazole in poultry

Animal	Purpose	Maximum dose rate	Critical comments
Turkeys, chickens	For treatment of blackhead (Histomoniasis)	**Medicated drinking water** Provide medicated water as the only source of drinking water for 12 days. Treat birds with 500 mg DMZ*/L water for the first 3–6 days, then treat with 250 mg DMZ/L water for the balance of the 12 days.	Medication for birds should be withdrawn during periods of high environmental temperature, where thirst may increase, or where dehydration may occur.
Turkeys	For treatment and/or prevention of blackhead	**Medicated feed** *Treatment:* Provide in-feed at a rate of 500 mg DMZ/kg feed for 7–14 days. *Prevention:* Provide in-feed continuously at a rate of 125 mg DMZ/kg feed.	
Chickens	For treatment and/or prevention of blackhead	**Medicated feed** *Treatment:* Provide in-feed at a rate of 500 mg DMZ/kg feed for 7–14 days then continue medication at the preventative level. *Prevention:* Provide in-feed continuously at a rate of 75 mg DMZ/kg feed.	

*DMZ = dimetridazole

Withholding periods on existing labels are:

MEAT: DO NOT ADMINISTER later than 5 days before slaughter for human consumption.

EGGS: DO NOT USE in birds which are producing, or may in the future produce, eggs or egg products for human consumption.

5.3.1. Residues history of dimetridazole in poultry

Between July 1971 and August 1989, the then Pesticides and Agricultural Chemicals Standing Committee (PACSC) of the National Health and Medical Research Council held a series of meetings to consider the residues of dimetridazole in meat of poultry, turkeys and pigeons. The PACSC concluded that there was a special requirement for the use of dimetridazole in breeders, and that the drug should be restricted to use in turkeys*, pigeons and poultry breeders. The assumption was that eggs from breeder birds would not be made available for human consumption, and so an egg MRL was never established.

* Turkey eggs are not used for human consumption.

5.4. Metabolism and analytical methodology considerations

The metabolism of dimetridazole in rats, pigs and turkeys was considered, as were the analytical methods used to determine levels of dimetridazole and its analytes in poultry eggs, and in meat from poultry and pigs.

5.4.1. Metabolism of dimetridazole in rats

In an unpublished study (Heijbroek 1976) evaluated only by JECFA, it was reported that when ^{14}C-dimetridazole was orally administered to rats, it was rapidly absorbed and excreted. The parent drug was extensively metabolised, with the successive formation of 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) and 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA), which were excreted in the urine. The pattern of dimetridazole metabolism in rats was reported to be similar to that observed in pigs and turkeys.

5.4.2. Metabolism of dimetridazole in pigs

Dimetridazole was rapidly absorbed in pigs following a single oral dose. About 40–60% of the administered dose was excreted within 24 hours of treatment (~75% in urine and 25 % in faeces). By seven days post-treatment, excretion had increased marginally to 40–70% (50–75% in urine and 25–50% in faeces). In expired air samples, about 3.3% and 4% of the administered dose, respectively, were recovered within 24 hours and seven days of dosing.

In one study (cited in JECFA 1989), analysis of the urinary residues excreted within 24 hours of dosing showed that 50–65% of the residues were made up of 5-nitroimidazole compounds, that is, the parent dimetridazole and its metabolites containing intact nitro groups. In a second study, chromatographic examination of urine from pigs collected during the first eight hours after dosing revealed the presence of dimetridazole (0.2% of total urinary activity), HMMNI (0.7%) and MNICA (18.7%). Conjugation of metabolites was not found to be a major pathway in pigs. Much of the urinary radioactivity was found to be associated with simple, naturally occurring compounds, such as amino acids. The composition of the faecal residues was not determined.

When pigs were treated with a single oral dose of 19–37 mg ^{14}C-dimetridazole/kg bw and sacrificed at seven days post-treatment, the levels of radioactive residues in edible pig tissues were 0.15 to 0.5 mg equiv/kg in muscle; 0.20 to 0.56 mg equiv/kg in fat; 0.6 to 1.1 mg equiv/kg in liver and 0.4 to 1.1 mg equiv/kg in kidney (Unsworth 1972). These levels were significantly high.

In a second study (cited in JECFA 1989), pigs were sacrificed at six hours after treatment with a single oral dose of 29.8 mg ^{14}C-dimetridazole/kg bw. HMMNI was found to be the major identifiable component in the residues of muscle and kidney. The results are tabulated in Table 5.

Table 5. Identity and quantity of dimetridazole residues in edible pig tissues at six hours post treatment

Compound	Dimetridazole residues					
	Muscle		Kidney		Liver	
	mg/kg	% of TRRs*	mg/kg	% of TRRs	mg/kg	% of TRRs
Dimetridazole	0.04	0.5	0.18	0.5	0.01	0.07
HMMNI	3.56	40.2	10.31	25.7	0.09	0.5
MNICA	1.33	13.8	1.55	3.6	ND**	ND
Total	**4.93**	**54.5**	**12.04**	**29.8**	**0.10**	**0.57**

*TRR = total radioactive residues
*ND = not detectable

In a similar experiment (cited in JECFA 1989) in which a pig was dosed with 16.6 mg ^{14}C-dimetridazole/kg bw, the only metabolite detectable in muscle 17 hours after dosing was HMMNI, which represented approximately 10% of the tissue radioactivity, equivalent to ~0.04 mg/kg.

5.4.3. Metabolism of dimetridazole in turkeys

Dimetridazole was rapidly absorbed and excreted from turkeys following a single oral dose (Law *et al*, 1962). 30–80% of the administered dose was recovered from the excreta of treated turkeys within 24 hours of treatment, and 60–100% was recovered within 72 hours. About 90% of the residues in excreta were water-soluble (extracted with water and 0.1 M hydrochloric acid). Less than 2% of the administered dose was recovered in expired air samples.

The water soluble residues components identified in turkey excreta using a series of paper chromatographic systems were:

- a sulfate conjugate of dimetridazole, 1-methyl-5-nitroimidazol-2-ylmethyl hydrogen sulfate (44.4% of the total excreted drug)
- 1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA) (25.8%)
- 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) (9.4%)
- unchanged dimetridazole (3.4%)
- a glucuronide conjugate of a 5-nitroimidazole compound (8.8%). Positive identification of this metabolite was not possible in the absence of appropriate reference compounds
- a non-nitro compound (6.2%), which was postulated to be a derivative of 1,2-dimethyl-5-aminoimidazole.

A further non-nitro compound (comprising 4.7% of the total excreted drug) was detected on autoradiograms, but did not absorb ultra-violet (UV) radiation, which is indicative of a degraded imidazole ring.

The metabolite profile for dimetridazole residues in edible turkey tissues (and eggs) has not been fully explained. In one study in which turkeys were treated with a single oral dose of ^{14}C-dimetridazole, and sacrificed at 72 hours post-treatment, the levels of radioactive residues in edible tissues (kidney, liver, muscle and skin) were reported to be below the method LOQs of less than 0.03 mg/kg to less than 0.05 mg/kg. However, these results are not conclusive, since the assay methods were not validated, and they employed a benzene extraction step, which is unlikely to efficiently extract the water soluble residues components from tissues.

Nevertheless, the major biotransformation products of other drugs belonging to the 5-nitroimidazole class, for example dimetridazole analogues such as metronidazole and ipronidazole, are qualitatively the same in all species studied. Therefore, it is concluded that the metabolism of dimetridazole in turkeys is likely to be extensive, and would follow the same metabolic pathways as dimetridazole in pigs.

Overall, the metabolism of dimetridazole in turkeys is believed to include:

- oxidation at the 2-methyl group, to give the hydroxymethyl (HMMNI) and carboxylic acid (MNICA) metabolites. It is noted that binding of HMMNI to proteins is likely to result in persistent residues in edible tissues of treated animals
- reduction at the 5-nitro group to give an amino compound, which would undergo rapid degradation. The nitro group supposedly reduces stepwise, via nitroso and hydroxylamino intermediates, to the amine. These intermediates, particularly the hydroxylamino, can covalently bind to protein or DNA. This binding of chemical electrophiles to cellular macromolecules is accepted as a mechanism by which chemical carcinogens initiate the neoplastic process
- fission of the 5-nitroimidazole ring. It is noted that one postulated product of this breakdown process is acetamide, a known chemical carcinogen.

5.4.4. Analytical methods

Details of the polarographic method used to determine dimetridazole residues in edible poultry tissues and eggs during residues trials were provided as part of the residues reports. In principle, the polarographic method would measure the levels of dimetridazole and any metabolites containing the nitro group (including HMMNI and MNICA), since the method involves reduction of the nitro moiety to an amino group. However, the method is dependent on the efficiency with which these analytes are extracted from tissues using benzene.

For poultry tissues, samples were homogenised with water or buffer solution, then extracted with benzene. The benzene extract was partitioned against an acidified aqueous solution. The aqueous phase, containing the dimetridazole analytes, was (sometimes) cleaned up with carbon tetrachloride to remove fats and other compounds that interfere with polarography or was mixed with an aqueous alkaline solution. Subsequently, the aqueous extract was saturated with borax, then thoroughly deoxygenated with oxygen-free nitrogen. Finally, a polarogram was recorded (-0.2 V to -0.9 V versus a Ag/AgCl or Hg electrode), and the residues levels were determined using an external standard calibration curve.

In the case of eggs, the benzene extraction procedure was not used. Instead, the yolk and albumen from each egg was separated, and aliquots of each fraction were saturated with borax, deoxygenated, and polarograms were recorded.

Validation data for the polarographic method were provided for dimetridazole (linearity r^2 of 0.9990 over the concentration range 0.05 to 5.0 mg/L; recovery range of 68 to 130% from samples fortified with 0.1 to 2.5 mg/kg dimetridazole), but not for the metabolites. The method LOQ for tissues and eggs was reported to be 0.1 mg/kg.

Owing to the relatively high water solubility of HMMNI and MNICA, it is contended that the levels of these analytes in the benzene extract are likely to be low. Therefore, the polarographic method is not considered to be adequate for regulatory purposes, because it has not been fully validated and its LOQ of 0.1 mg/kg is not sufficiently sensitive.

A search of the published literature revealed that there are a number of analytical methods available to measure dimetridazole residues in edible tissues, all of which are more sensitive than the polarographic method used in the studies available to this review. Details of some of these methods are provided in Table 6.

Table 6. Validated analytical methods for the determination of dimetridazole residues

Method description	Reference (Section 11)	Analytes	Samples	LOQ/LOD (mg/kg)	Comments
*HPLC/UV detection	1	DMZ** HMMNI	Eggs	LOQ 0.005–0.010	
	16	DMZ	Poultry meat Pig meat	LOD 0.005 LOQ 0.010	
	47	DMZ HMMNI	Poultry meat Eggs	LOD 0.0005	Method used as part of the UK surveillance program.
	51	DMZ HMMNI	Turkey meat Pig meat	LOQ 0.001	Method used as part of the US surveillance program.
Liquid chromatography	48	DMZ HMMNI	Pig tissues	LOD 0.001–0.002	
HPLC with thermospray MS/MS***	30	DMZ HMMNI	Turkey tissues	LOQ 0.002	Method used to confirm identity of analytes detected with HPLC/UV method.
GC/NICI/MS	51	DMZ HMMNI	Turkey meat Pig meat	LOQ 0.001	Method used by the USA to confirm identity of analytes detected with the HPLC/UV method.
HPLC with atmospheric pressure chemical ionisation MS	47	DMZ HMMNI	Poultry meat Eggs	LOD 0.0001–0.0005	Method used by the UK to confirm identity of analytes detected with the HPLC/UV method.
HPLC with electrochemical detection	6	DMZ HMMNI	Pig tissues	LOQ 100–200 pg/sample	

Method description	Reference (Section 11)	Analytes	Samples	LOQ/LOD (mg/kg)	Comments
Gas chromatography	40	HMMNI	Pig muscle	LOD 0.001 LOQ 0.002	
High performance TLC**** with fluorescence detection	17	DMZ HMMNI	Pig meat Poultry meat	LOQ 0.005–0.010	

*HPLC = high pressure liquid chromatography
**DMZ = dimetridazole
***MS = mass spectrometry
****TLC = thin layer chromatography

Both the USA and the UK employ an HPLC method with UV detection for monitoring and compliance purposes. The methods determine the levels of dimetridazole and its hydroxy metabolite (HMMNI) in edible tissues and eggs, and have an LOQ of 0.001 mg/kg.

5.4.5. Residues definition

The existing Australian residues definition for dimetridazole in Table 3 of the MRL Standard consists of the parent compound only. This residues definition is not considered to be appropriate, since the results from metabolism studies have shown that the hydroxy metabolite (HMMNI) is the major component of residues in edible tissues from treated animals. Suitable analytical methods are available to measure residues of both dimetridazole and HMMNI in edible tissues and eggs down to concentrations of 0.001 mg/kg.

As a result, the APVMA decided that the residues definition for dimetridazole be amended to:

> The sum of dimetridazole and its hydroxy metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole, expressed as dimetridazole.

5.5. Residues data and risk assessment methodology

Table 1 in the MRL Standard currently lists the MRLs for dimetridazole in pig and poultry meat and edible offal of pigs and poultry as 0.005 mg/kg. However, with the withdrawal of the ADI and the lack of a suitable database to establish an ARfD for dimetridazole, all commodities from treated animals must have nil residues if they are to be made available for human consumption.

The residues assessment was based on the existing available residues data for poultry, and took into account the fact that edible commodities from breeder birds could conceivably enter the human food chain when:

- the supply of fertilised eggs exceeds the hatchery requirements. The excess eggs may be sold for human consumption
- replacement pullets/breeders are culled. Meat from these birds may be sold or processed for human consumption
- breeder hens have reached the end of their economic lifespan ('spent hens'). Meat from these birds may be sold or processed for human consumption.

In order to consider the residues arising from the use of dimetridazole on broiler breeders and turkey breeders, the available residues data for poultry tissues and eggs were reviewed.

5.5.1. Dimetridazole residues in eggs

The Chemistry and Residues Program considered data from a single residues trial (Amis *et al*, 1964) in which laying hens were orally dosed with 125, 250 or 500 mg dimetridazole/kg feed for three consecutive weeks. Eggs were collected for up to six days following withdrawal of the medicated feed, and residues were determined directly in the albumen and yolk fractions using the polarographic method described in paragraph 5.4.4. The residues results were corrected to reflect the maximum 1× label rate of 500 mg dimetridazole/kg feed (see Table 7).

Table 7. Dimetridazole residues in eggs—corrected to the maximum 1× label rate (500 mg dimetridazole /kg feed)

Sampling time (days after last treatment)	Parameter	Dimetridazole residues (mg/kg)		
		Albumen	Yolk	Whole egg (minus shell)
0	Range	4.0 – 8.4	1.2 – 7.8	3.2 – 6.7
	Mean ± SD*	5.6 ± 1.2	4.5 ± 1.8	5.1 ± 1.0
	Upper 95% CL**	7.9	8.0	7.1
	n	14	14	14
1	Range	3.2 – 7.3	1.6 – 9.2	2.4 – 6.9
	Mean ± SD	4.8 ± 1.1	4.2 ± 1.7	4.6 ± 1.2
	Upper 95% CL	7.0	7.6	6.8
	n	22	22	21
2	Range	0.2 – 0.9	0.8 – 4.6	0.6 – 2.0
	Mean ± SD	0.6 ± 0.2	1.4 ± 1.0	0.9 ± 0.4
	Upper 95% CL	1.1	3.4	1.7
	n	13	13	13
3	Range	0.2 – 0.4	0.3 – 0.5	0.2 – 0.4
	Mean ± SD	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
	Upper 95% CL	0.4	0.5	0.5
	n	7	7	7
4	#Range	<0.1 – 0.8	<0.1 – 0.4	<0.1 – 0.6
	Mean ± SD	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
	Upper 95% CL	0.6	0.2	0.4
	n	19	19	19
5	#Range	<0.1 – 0.1	<0.1 – 0.1	<0.1 – 0.1
	Mean ± SD	<0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1
	Upper 95% CL	0.1	0.1	0.1
	n	8	8	8
6	#Range	<0.1	<0.1	<0.1
	Mean ± SD	<0.1 ± 0	<0.1 ± 0	<0.1 ± 0
	Upper 95% CL	<0.1	<0.1	<0.1
	n	6	6	6

*SD = standard deviation
**CL = confidence limit
Values recorded as <0.1 mg/kg were taken as 0.05 mg/kg for statistical purposes.

Since the polarographic method used to measure dimetridazole residues in eggs did not incorporate a benzene extraction procedure and the egg yolk and albumen fractions were assayed directly, the residues results are likely to have quantified the levels of all dimetridazole residues components containing an intact nitroimidazole ring. As such, the results did not precisely correspond to the proposed residues definition for dimetridazole of parent dimetridazole plus the hydroxy metabolite, HMMNI. In fact, the results are likely to over-estimate the residues levels that would be detected using an appropriate regulatory method. Nevertheless, the results from the egg trial provided useful information on the residues decline profile in eggs, and may be extrapolated to estimate when a 'nil residues' situation is reached.

The highest residues were observed in eggs collected immediately following the cessation of treatment or within one day of drug withdrawal. The maximum reported residues levels were 8.4 mg/kg in albumen, 9.2 mg/kg in egg yolk, and 6.9 mg/kg in whole egg (minus shell). At no time did the highest residues occur in the albumen and yolk of a single egg, which explains why the maximum residues observed in whole egg are less than those observed for both the albumen and yolk fractions.

Total dimetridazole residues in eggs declined rapidly, with eggs produced on the second day of drug withdrawal reported to have maximum levels of 0.9 mg/kg in albumen, 4.6 mg/kg in yolk and 2.0 mg/kg in whole egg. Residues in yolk, albumen and whole egg were reported to be below the LOQ of 0.1 mg/kg within six days of drug withdrawal.

At the time this assessment was conducted, the rationale was that the use of dimetridazole in breeder poultry could be justified from a human health perspective, if the withholding period was lengthened to ensure that there were no dimetridazole residues in excess eggs and meat from culled or spent breeder birds. Exposure of consumers to dimetridazole residues in these commodities was considered to be nil, since the residues concentrations in food would be nil.

The length of the withdrawal period required for egg residues to become zero was estimated by:

- determination of the upper 95% confidence limits at each sampling time, and extrapolation of the regression lines for the decline data, out to 'zero' residues
- calculation of the number of half-life periods required for the residues in eggs to decline to 'zero', and multiplying this figure by the half-life estimate for dimetridazole residues in eggs.

When calculating the withdrawal period estimates, the 'nil residues' level was initially defined as the LOD of the most sensitive analytical method that can be used to quantify dimetridazole residues in eggs and tissues. This value was 0.0005 mg/kg.

Statistical analysis of the egg residues data revealed that dimetridazole residues in albumen are expected to decline (with 95% confidence) to less than 0.0005 mg/kg within nine days of the cessation of treatment. Similarly, residues in yolk were expected to be less than 0.0005 mg/kg within 11 days. Thus, dimetridazole residues in eggs were likely to be 'nil' within 14 days of drug withdrawal.

Dimetridazole residues in albumen had an estimated half-life of about 0.5 days, and residues in yolk had an apparent half-life of about 0.7 days. Thus, when a conservative half-life of one day was used, and the maximum dimetridazole residues in whole eggs were taken to be 10 mg/kg, then residues would have declined to less than 0.0005 mg/kg within 15 half-lives (equivalent to 15 days).

The estimated 14-day period for egg residues to decline to non-detectable is consistent with the physiological time frame for egg development. Egg follicles develop rapidly in about the last 10–14 days before ovulation, increasing in size from about 1 gram to about 20 grams. During this development phase, drug residues are deposited in concentric rings as the yolk is laid down. Therefore, yolks in eggs that are laid 14 days after the cessation of treatment are unlikely to have been rapidly developing during the treatment phase.

However, the Chemistry and Residues Program noted that the egg withdrawal period estimates were based on a limited data set of one residues study that was conducted using an un-validated analytical method, and that the method has a relatively high LOQ that necessitated extensive extrapolation. Therefore, at the time, the APVMA considered it appropriate to apply a safety factor to the egg withdrawal period estimates, and set the egg withholding period at 28 days. Using this approach, the corresponding 'nil residues' level was estimated to be in the order of 10^{-8} mg/kg, that is, 0.01 parts per trillion. The practicality of this approach is discussed further in paragraphs 5.5.3 and 5.5.4.

5.5.2. Dimetridazole residues in poultry edible tissues

The Chemistry and Residues Program evaluated a series of residues trials conducted in turkeys (Ward 1964, Muggleton 1965, *Residues of dimetridazole and amprolium in turkey tissues after separate and combined administration* 1965) and chickens (Muggleton 1963). The results from these trials indicated that when birds are treated at the maximum label rate of 500 mg dimetridazole/kg feed or 500 mg dimetridazole/L drinking water, residues in edible tissues decline to below the LOQ (less than 0.1 mg/kg) within two days of drug withdrawal. However, the data from these trials were of limited value, since a polarographic method that incorporated a benzene extraction step was used to assay the levels of dimetridazole residues in edible tissues from treated birds.

The validity of the residues results is dependent on the efficiency with which the residues components are extracted from tissues using benzene. Owing to the relatively high water solubility of the residues components, it is likely that the extraction efficiency was poor. Consequently, the residue data were unlikely to accurately reflect the levels of incurred residues in edible tissues.

In the absence of appropriate residues data for edible tissues, the Chemistry and Residues Program extrapolated a meat withholding period from the residues data for eggs. The distribution of residues into tissues, and subsequent residues depletion over time, is a function of the drug pharmacokinetics.

During treatment, the drug circulates through the body, and partitions into tissues. During the elimination phase, the drug is metabolised and/or excreted, with tissue residues being remobilised then re-entering the blood.

Given that the albumen fraction of eggs is produced and excreted in the 24 hours before laying, the presence of any dimetridazole residues in plasma would be expected to result in the deposition of residues in albumen. Thus, when egg albumen residues are determined to be zero, after a 28-day withdrawal period, the levels of tissue residues are also expected to be zero. On the basis of this rationale, the meat withholding period could be set at 28 days. See next paragraph (5.5.3) for further discussion on setting a withholding period for dimetridazole.

5.5.3. Consideration of the risk assessment methodology initially used

In the past, the Australian practice of setting MRLs at or about the method LOQ for chemicals that do not have an ADI or ARfD was considered acceptable. However, in light of contemporary risk assessment methodologies, this practice has raised questions and concerns locally as well as internationally.

In the original risk assessment, the Chemistry and Residues Program assumed that, in the absence of an ADI of ARfD, the public health standard for dimetridazole was the LOQ of the available analytical method. But the sensitivity of the analytical method, the LOQ, is a function of the sample preparation procedure and the analytical instrumentation. For example, the method LOQ for dimetridazole residues studies generated 30 years ago was 0.1 mg/kg (100 ppb). Present-day methods for analysing dimetridazole have an LOQ of 0.0001 mg/kg (0.1 ppb). This represents a 1000-fold increase in method sensitivity.

The LOQ has no correlation to toxicological No-Observed- Adverse Effect Levels, the biological measures used to establish the health intake standards. Therefore, it is invalid to use the LOQ as a pseudo health standard.

The initial residues risk assessment (see paragraphs 5.5.1 and 5.5.2) tried to retain some uses of dimetridazole. However, it is not possible to set MRLs without an ADI, as there is no exposure level that can satisfy public health requirements.

Withholding periods for a drug are set on the basis of the residues decline profile of that drug. For dimetridazole, the withholding period needs to be long enough to allow residues to decline to below the present-day method LOQ of 0.0001 mg/kg. However, available data considered in the review only monitor the decline of dimetridazole residues down to 0.1 mg/kg, which necessitates extensive extrapolation well beyond the actual data. The lack of empirical data for the residues decline profile between 0.1 mg/kg and 0.0001 mg/kg means it is not possible to establish a suitable withholding period to achieve 'nil' residues.

Consequently, it is concluded that the APVMA cannot be satisfied of its legislative requirements that the use of dimetridazole in food-producing species would not be an undue hazard to human health.

5.5.4. Dietary risk assessment

Dietary intake assessments involve calculation of the dietary exposure levels (amount of treated food consumed × residues level), and comparison of these exposure levels with the ADI for chronic dietary exposure, and with the ARfD for acute dietary exposure. An intake less than 100% of the ADI/ARfD is generally considered to be acceptable. In contrast, an exposure level significantly greater than 100% is considered to present an unacceptable risk to human health.

Initially, the dietary risk to human health from the use of dimetridazole in broiler breeders and turkey breeders was considered to be nil. However, since the Department of Health and Ageing has withdrawn the former ADI for dimetridazole, the Chemistry and Residues Program is not able to conduct an acute or chronic dietary intake risk assessment for dimetridazole residues in foodstuffs.

Furthermore, the existing residues definition for dimetridazole does not include the metabolites, particularly, the hydroxy metabolite. This metabolite is toxicologically significant, and may be present in foods at twice the concentration of parent dimetridazole.

When current APVMA and international risk assessment methodologies are applied, the Chemistry and Residues Program is not able to establish a safe consumption level for dimetridazole in the absence of an ADI or ARfD. Consumption of commodities containing residues at levels below the method LOQ from the residues studies available to the Chemistry and Residues Program may potentially constitute an undue risk to human health. When the factors outlined in this paragraph are considered together with the potential genotoxic carcinogenicity concerns about dimetridazole , the APVMA can no longer support the use of dimetridazole in breeder poultry.

5.5.5. Residues-related aspects of trade

Risks to Australian trade from chemical residues arise when importing countries have not set tolerances for the chemical/drug residues in food commodities, or when tolerances in the importing countries are lower than the corresponding Australian MRLs.

Dimetridazole is classified as a 'banned substance' in the USA and EU. It is listed in section 530.41 of the USA Code of Federal Regulations – 'substances that are prohibited for extra-label animal and human drug uses in food-producing animals'. In the EU the nitroimidazoles are Group A substances which are prohibited from use in food-producing species. This means that commodities from animals treated with dimetridazole at any time during their life cannot enter the export market, even if residues are not detectable. In Volume II of the Australian Quarantine and Inspection Service (AQIS) Export Meat Manual, the Essential Country Requirements for the USA state 'Pig meat to the USA must be declared free of dimetridazole by the producer, to the effect that dimetridazole has never been used in the treatment of pigs from which the product is sourced'.

Although the USA and EU are not Australia's major trading partners in poultry and pork commodities, the trade requirements of these countries are commonly referenced by importing countries that have not established their own tolerance standards.

6. OVERSEAS REGULATORY STATUS

United States

In 1988, the US EPA announced the withdrawal of approvals for dimetridazole. The regulators in the United States considered that dimetridazole was demonstrated to have mutagenic effects on several bacterial strains and *Drosophila melanogaster* (fruit flies); that it caused a significant increase in the occurrence of mammary tumors in rats; that it is chemically similar to ipronidazole and metronidazole, chemicals that are considered to be carcinogenic in a mammalian species (mice); and that several metabolites of dimetridazole are suspected carcinogens. In addition, the US EPA considered that the available studies were inadequate (in terms of standards for such testing, including the number of animals tested) and that there was an unacceptably high level of use of the chemical off-label in food-producing animals. Dimetridazole was not approved in the United States for use in pigs. The United States has continued to allow the use of dimetridazole under veterinary prescription, provided such use is confined to non-food-producing animals.

European Union

In July 1995, the EU withdrew the authorisation of dimetridazole as a veterinary medicine over concerns about its potential carcinogenicity. Although dimetridazole continued to be approved for use as a feed additive in the EU until 2001 the EU banned its use as a feed additive with effect from May 2002 on the basis that insufficient data had been submitted to meet the requirements of the re-evaluation. Dimetridazole continued to be used under licence as a feed additive in game birds in the United Kingdom until 2002, when supply was discontinued by the only registrant responsible for supply in the United Kingdom in response to the uncertain status of the chemical.

Canada

The Veterinary Drugs Directorate of Health Canada published amended regulations in August 2003 that withdrew the authorisation of all 5-nitroimidazole chemicals for use in animals that produce food for human consumption. The reasons given for this action related to the lack of sufficient toxicological information for bound residues of metabolic products. The Veterinary Drugs Directorate continues to support the use of 5-nitroimidazoles in non-food-producing animals. The regulatory impact analysis statement released by Health Canada specifies that the use of 5-nitroimidazoles is permitted in some species, including ‘poultry birds’, provided adequate records are kept of treated animals and the animals are not sold for human consumption. The use of 5-nitroimidazoles in Canada is not permitted in pigs as all swine are considered to be food-producing animals, whereas poultry birds can be food-producing animals or non-food-producing animals according to Health Canada.

JECFA

The Joint Expert Committee on Food Additives (JECFA), a joint body of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), reported that it was unable to set an ADI for dimetridazole when it considered the chemical in 1989. JECFA reported examining a study that showed an increase in the incidence of tumors in rats exposed to dimetridazole. By April 2002 JECFA reported that, in addition to inadequacies in the available data (in terms of standards for such testing), there was also insufficient information available to support the setting of an ADI or an MRL for dimetridazole due to a lack of data establishing the relative toxicological potency of bound residues and the absence of additional data establishing the relationship between a marker compound and the total residues.

In June 2002, JECFA concluded that dimetridazole is mutagenic *in vitro* but that available evidence is insufficient to determine if dimetridazole is mutagenic in animals. JECFA therefore requested that specific additional studies be undertaken to determine if dimetridazole is mutagenic in animals, and placed the chemical on the inactive list pending receipt and evaluation of additional human safety data.

New Zealand

The Agricultural Compounds and Veterinary Medicines Group of the New Zealand Food Safety Authority included dimetridazole in a list of restricted substances and veterinary medicines in response to trade risks. Where a restricted veterinary medicine is used on food animals, conditions are imposed on the product registration unless tagging and tracking programs are instituted. A concern of New Zealand is the potential for off-label use of dimetridazole within the ostrich industry. The Animal Products Group of the New Zealand Food Safety Authority intended to address this concern by imposing additional restrictions on the overseas market access requirements for ostriches.

7. SUMMARY OF PUBLIC SUBMISSIONS

7.1. Comments received during review scoping

Eleven written submissions were received from state government departments, registrants and user groups when the scope of the review was considered. The concerns outlined in the submissions varied from repeated observations that there is no suitable alternative chemical treatment available to control blackhead in poultry, particularly turkeys, with the consequent potential for significant adverse economic impact on a range of business interests, to the potential impact on human health, including worker safety, particularly at the level of the end user.

Two submissions were received from state government departments stating that products containing dimetridazole are considered essential in both the poultry and pig industries. Alternative chemical treatments are available for the control of swine dysentery in pigs but no therapeutic agent is available for the prevention or treatment of blackhead in poultry. It was also noted that careful management controls have helped to reduce the occurrence of outbreaks of both these diseases but that outbreaks are a serious problem when they occur.

Four submissions from producer organisations argued that dimetridazole is an important chemical in the production of chicken and turkey meat in Australia. One submission included an estimate of the current economic impact from recent occasional outbreaks of blackhead. The information supported the view of producer groups that there is likely to be a significant economic impact if the use of dimetridazole in chickens and turkeys is discontinued.

One submission indicated that there is an important off-label use of products containing dimetridazole to treat canker, caused by *Trichomonas spp.*, in meat (squab) pigeons. The submission indicated that dimetridazole is primarily used in young birds kept for breeding purposes, and that the loss of dimetridazole for this use could have serious repercussions for the industry.

7.2. Comments received during the public consultation period

After the draft review report was released, 21 submissions were received from state government departments, user/producer groups, industry peak bodies and veterinarians.

Three submissions commented that the terminology and instructions proposed in the draft report were confusing and contradictory. Statements with clearer meanings were suggested.

Twelve submissions widely supported on-going use of dimetridazole in broiler breeder and turkey breeder flocks. There was some support for the use of dimetridazole in replacement pullet flocks and grower meat turkeys if residues monitoring was implemented. Two of the submissions indicated that additional data would not be obtained to support any use in egg layer pullets.

Four veterinary poultry consultants offered assurances that a long withholding period and restrictions on the use of dimetridazole could be managed through prescriptions.

Another four submissions answered questions relating to the structure of the broiler breeder and turkey breeder industry, management practices, production processes, industry controls in place, and frequency and management of blackhead outbreaks.

Four submissions supported the use of dimetridazole to eradicate swine dysentery from pig farms. The pig industry stated that it is feasible for producers to observe a withholding period and an export slaughter interval of 28 days for pig meat and offals and argued that quality assurance systems, initiatives for monitoring residues of dimetridazole, and identification and tracking of individual animals back to the property of origin were already in place.

The pig and poultry industries both argued that the non-availability of dimetridazole would impact negatively on health, production and welfare of valuable breeding pigs and poultry. One submission stated that the economic cost through illness outweighed the risks of using dimetridazole with a 28-day withholding period.

Three of the submissions questioned the adequacy of the risk assessment methodology that was used in the residues assessment. Information was received on the sensitivity of available analytical methods for dimetridazole.

8. FINDINGS AND CONCLUSIONS OF THE REVIEW

8.1. Scheduling, safety directions and first aid instructions

Dimetridazole is currently listed in Schedule 4 of the SUSDP. The review notes that the current entry will remain, in the event that registered veterinary products continue to be made available for non-food-producing birds. Furthermore, new safety directions are recommended (see Section 9.2 of this report) for dimetridazole while the current first aid instruction entry in the Handbook of First Aid Instructions and Safety Directions (FAISD Handbook) is retained.

8.2. Use of dimetridazole in food-producing species

Available data suggested that dimetridazole is a potential genotoxic carcinogen. The review notes the OCS finding that there are insufficient toxicology data to support the ADI that was established in 1986. As a result, the ADI entry for dimetridazole has been deleted from the Australian ADI list which is maintained by the Australian Government Department of Health and Aging, because there is no expectation of receiving new data to address the genotoxic carcinogenicity concerns about dimetridazole. Because the concerns about dimetridazole were not allayed, it is not safe to use the chemical in food-producing species. Therefore, the APVMA cannot be satisfied that the continued use of dimetridazole in food-producing species, such as pigs, chickens and turkeys, would not be likely to have an effect that is harmful to human beings. An ARfD for dimetridazole has not been established by OCS and is considered not applicable since the use of the chemical in food animals is not supported. Without an ADI for dimetridazole, the APVMA cannot conduct a risk assessment to determine a safe consumption level of dimetridazole in food commodities. Furthermore, without knowing what would be a safe consumption level of DMZ in food commodities, the APVMA cannot vary the conditions of registration and label approval of the products which have uses only in food-producing species such that the requirements for their continued registration and label approval are met.

Due to safety concerns about genotoxicity and the lack of data to address these concerns, the registration of dimetridazole products for use in food-producing animals is not supported and should be withdrawn.

8.2.1. Use in pigs

All pigs, including breeding stock, are generally intended for human consumption. The APVMA considered the possibility of retaining the use of dimetridazole in pigs, including breeding sows, for the eradication of swine dysentery. In such a case, a condition of registration would be that meat and offal from dimetridazole-treated pigs must not enter the human food chain at any time during their life, even if residues are not detected. The pig industry would have been required to demonstrate that they have the ability to ensure that carcasses from treated sows do not enter the human food chain at any time after treatment. However, upon further consideration, it was identified that piglets born to dimetridazole-treated sows may be exposed to dimetridazole *in utero.* Thus, these piglets would not meet the export trade requirement that they have never been exposed to dimetridazole during their lifetime.

The review concludes that the continued use of dimetridazole in breeding sows is not supported.

8.2.2. Use in chicken layer hens

Most products containing dimetridazole state on their label's withholding period instructions that the products should not be used in birds that are producing eggs, or may in the future produce eggs, for human consumption. However, a submission from egg producers indicated that at least one product containing dimetridazole may have been used in layers up to the point of lay. Historically, dimetridazole was never registered in Australia for use in poultry that lay eggs for human consumption. Therefore, eggs containing residues of dimetridazole are unsuitable for human consumption.

The use of dimetridazole in layer hens that are producing eggs for human consumption, or may in the future produce eggs for human consumption is not supported.

8.2.3. Use in breeder chickens and breeder turkeys

There is currently no MRL entry in the MRL Standard for dimetridazole in eggs. The APVMA had considered the possibility of retaining limited use of dimetridazole in breeder chickens and breeder turkeys. In the present position, a condition of registration would be that eggs and meat from dimetridazole-treated birds must not enter the human food chain at any time during their life, even if residues are not detected. The poultry industry would be required to demonstrate that they have the ability to ensure that excess eggs and meat carcasses from spent hens or culled replacement pullets do not enter the human food chain at any time after treatment. Upon further consideration, it was identified that birds hatched from eggs laid by dimetridazole-treated breeder birds could not meet the requirement that they have never been exposed to dimetridazole during their lifetime.

In the absence of data to address the genotoxic carcinogenicity concerns about dimetridazole to the extent that an ADI or ARfD for dimetridazole cannot be established, the continued use of dimetridazole in breeder chickens and breeder turkeys is not supported.

8.3. Uses in non-food-producing species

From a toxicological perspective, the draft review report had stated that ongoing use of dimetridazole in pigeons, caged birds and game birds was supportable, provided meat and eggs from these species were not made available for human consumption, and provided that labels were varied with the addition of safety instructions. However, the APVMA is aware that squab pigeons and game birds are intentionally reared for human consumption. Furthermore, there are concerns that if dimetridazole is made available for use in pigeons, caged birds and game birds not intended for human consumption, it might be used off-label in food-producing birds. The potential for off-label uses would constitute a risk to both human health and Australia's export trade, but this risk can be managed by the inclusion of restraint statements on the label. Through this measure, the APVMA can be satisfied that the continued use of dimetridazole in non-food-producing species would not be likely to have an effect that is harmful to human beings.

Thus, the continued use of dimetridazole in pigeons, caged birds and game birds is supported with a restriction on the chemical being administered to birds destined for human consumption.

8.4. OHS risk mitigation

Both the APVMA and OCS had regard to the issues raised in the public submissions. Veterinarians and other product users contacted the APVMA following the announcement of the review. Their concerns about the lack of OHS information on product labels, and the general absence of information about dimetridazole as a possible carcinogen, are addressed by the inclusion of safety directions on labels. Users could seek additional information from Material Safety Data Sheets.

Although dimetridazole poses a genotoxic concern, the recommended safety directions (see Section 9) are considered sufficient to reduce the potential risks to workers from acute and repeated exposure to the chemical.

The draft review report indicated that exposure to dimetridazole powder/dust could be further reduced if registrants were to package their products in water-soluble sachets. This type of packaging would negate the need for users to wear a respirator. If these risk reduction measures are adopted, the APVMA can be satisfied that the continued use of dimetridazole in non-food-producing animals in accordance with new, varied label instructions, would not be an undue hazard to the safety of people exposed to the chemical during its handling.

9. REGULATORY APPROACH

The APVMA finds that the continued use of the products listed in Appendix B in accordance with the instructions for their use:

- would be an undue hazard to the safety of people exposed to them during their handling or use
- would be likely to have an effect that is harmful to human beings.

On these grounds, the APVMA is not satisfied that the criteria for continued registration and for continued label approval are met.

However, the APVMA finds that the conditions of label approval for the products listed in Appendix D can be varied in accordance with s.34(5) of the Agvet Code in the manner described in section 9.1 of this report, such that the criteria for continued label approval are met.

On these grounds, the APVMA is satisfied that the continued registration of the products in Appendix D when used in accordance with new, varied instructions:

- would not be likely to have an effect that is harmful to human beings
- would not be an undue hazard to the safety of people exposed to them during their handling or use.

9.1. Variation of the conditions of registration and approvals

The APVMA decided to vary the current labels for the products in Appendix D as follows:

- uses of dimetridazole in pigs, chickens and turkeys are deleted
- uses of dimetridazole in breeder pigeons, caged birds and game birds not destined for the human food chain are retained
- directions for use are updated with restraint statements, warning statements and dosage and administration instructions as outlined in section 9.4 of this report
- safety directions and first aid instructions are updated.

Because labels for containers of products in Appendix D are varied as described, the APVMA decided to affirm the registrations and label approvals of these products. As label approval 50743/1200 is not current, the APVMA decided to cancel this approval.

9.2. Cancellation of registrations and label approvals

The APVMA finds that it is not satisfied that the conditions of registration and label approval of the products in Appendix C can be varied in such a way that the requirements for continued registration and label approval will be complied with.

On this basis the APVMA decided to cancel the registration and label approvals of products listed in Appendix C.

9.3. Phase-out provisions

The APVMA will not recall any batches of cancelled products and labels. The APVMA will allow the possession, supply and use of existing batches manufactured prior to the APVMA Board of Directors' final regulatory decision, for 24 months from the date of that decision. During this period, wholesale and retail supply of existing stocks of dimetridazole will be permitted. However, if manufacturers are unable to ensure that their cancelled products are supplied with the instructions outlined in section 9.4, the APVMA will issue a notice of recall to those manufacturers.

The manufacture of new batches of product and the importation of new consignments of dimetridazole active ingredient will be prohibited from the date of the Board's decision. Persons who have ordered new consignments of dimetridazole active ingredient prior to the Board's decision may apply to the APVMA for a Consent to Import Certificate if the consignment is still in transit on the date of the Board's final regulatory decision.

End-users will be permitted to use existing batches of the cancelled products in accordance with the instructions outlined in section 9.4.

9.4. Label instructions for phase-out period

The APVMA will issue the following new withholding periods and instructions on how the cancelled products are to be used during a phase-out period. The instructions and withholding periods are expressed in accordance with the Vet Labelling Code.

Indications

For the treatment and prevention of blackhead caused by *Histomonas meleagridis* in broiler breeders, layer breeders, turkey breeders and breeder game birds.

For the treatment and prevention of swine dysentery caused by *Brachyspira hyodysenteriae* in breeding pigs.

For the treatment of canker caused by *Trichomonas gallinae* in breeder pigeons and breeder caged birds.

Directions for use

Restraint

DO NOT USE in chicken layer hens and cockerels, turkey layer hens and toms, broiler chickens and grower (meat) turkeys.

DO NOT USE in pigeons, caged birds and game birds intended for human consumption.

DO NOT USE this product to treat any animal species, or category of animal, not included in the Dosage and Administration table.

DO NOT supply this product except in the original, unopened packaging.

DO NOT re-treat breeder chickens, breeder turkeys and breeding pigs for 28 days after the last treatment period.

Warning

Dimetridazole may cause genetic damage in users.

Dosage and Administration

Host Animal	Purpose	Maximum Dose Rate
Broiler breeders, layer breeders and turkey breeders	For treatment of blackhead.	**Medicated drinking water** Provide medicated water as the only source of drinking water for 12 days. Treat birds with 500 mg dimetridazole/L water for the first 3-6 days then treat with 250 mg dimetridazole/L water for the balance of the 12 days.
Turkey breeders	For treatment and/or prevention of blackhead (Histomoniasis)	**Medicated feed** *Treatment*: Provide in-feed at a rate of 500 mg dimetridazole/kg feed for 7-14 days. *Prevention*: Provide in-feed continuously at a rate of 125 mg dimetridazole/kg feed.
Broiler breeders and layer breeders	For treatment and/or prevention of blackhead	**Medicated feed** *Treatment*: Provide in-feed at a rate of 500 mg dimetridazole/kg feed for 7-14 days then continue medication at the preventative level. *Prevention*: Provide in-feed continuously at a rate of 75 mg dimetridazole/kg feed.
Breeding pigs	For treatment and prevention of swine dysentery	**Medicated feed** *Prevention*: Provide in-feed at a rate of 200 mg dimetridazole/kg feed. *Treatment*: Provide in-feed at a rate of 900 mg dimetridazole/kg feed. **Medicated drinking water** *Prevention*: Provide in-water at a rate of 250 mg dimetridazole/L water for 3-7 days.
Pigeons and caged birds	For treatment of canker	**Medicated drinking water** Treat birds with 250 mg dimetridazole/L water for 3-7 days
Game birds	For treatment of blackhead	**Medicated drinking water** *Treatment*: Provide medicated water as the only source of drinking water for 12 days. Treat birds with 500 mg dimetridazole/L water for the first 3-6 days then treat with 250 mg dimetridazole/L water for the balance of the 12 days

Withholding Periods

Poultry

Meat: Meat from culled breeder replacement hens, cockerels and spent breeder hens must not be made available for human consumption for 28 days after the end of treatment.

Eggs: DO NOT USE in poultry layer hens that are producing or may in the future produce eggs or egg products for human consumption. Excess eggs from breeder hens must not be made available for human consumption for 28 days after the end of treatment.

Pigs

Meat: DO NOT administer less than 28 days before slaughter for human consumption.

Safety directions

When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge). After each day's use wash contaminated clothing, gloves and respirator, and if rubber, wash with detergent and warm water.

Or for products containing dimetridazole and calcium lignosulfonate 20 g/kg or greater, the following safety directions apply:

Repeated exposure may cause allergic disorders. When opening the container or preparing the mix, wear chemical resistant overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, chemical resistant footwear and a half-facepiece respirator (with dust cartridge). After each day's use wash contaminated clothing, gloves and respirator, and if rubber, wash with detergent and warm water.

First aid instructions

If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.

9.4.1. Other label information

Information relating to the signal heading block, product name, active constituent, contents, disposal, storage, APVMA Approval Number, batch number and expiry date are specific for individual products and are to be retained during the phase-out period.

As the expiry date of product batches will vary depending on the date of manufacture, during the phase-out period users would be allowed to use the products for 24 months after the date of cancellation or up to the expiry date of the products, whichever occurs first.

9.5. Amendments to standards

The APVMA decided to amend the residues definition and the MRLs in the MRL Standard of Australia. These changes are necessary to facilitate the phase out of dimetridazole and will further limit the occurrence of dimetridazole residues in edible commodities during the phase-out period.

9.5.1. Residues definition

The existing Australian residues definition for dimetridazole consists of the parent compound only. This residues definition is not considered to be appropriate, since the results from metabolism studies have shown that the hydroxy metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI), is the major component of residues in edible tissues from treated animals. Furthermore, there are suitable analytical methods available to measure residues of dimetridazole and HMMNI in edible commodities down to concentrations of 0.0001 mg/kg (0.1 ppb).

The APVMA decided that the residues definition for dimetridazole in Table 3 of the MRL Standard be amended to the **sum of dimetridazole and its hydroxy metabolite (2-hydroxymethyl-1-methyl-5-nitroimidazole), expressed as dimetridazole**.

9.5.2. Maximum Residue Limits

Based on the available residues decline data for pigs and poultry, the concentrations of dimetridazole residues in edible commodities are expected to be well below the method LOQ of 0.0001 mg/kg when 28-day withholding periods are observed for meat and eggs. Therefore, for the duration of the dimetridazole label phase-out period, it is recommended that temporary dimetridazole MRLs of *0.0001 mg/kg be established for all pig and poultry commodities, including a new entry for poultry eggs.

Amendments to Table 1 of MRL Standard

Compound	Food	MRL (mg/kg)
Dimetridazole		
DELETE		
MO 0818	Pig, Edible offal of	*0.005
MM 0818	Pig meat	*0.005
PO 0111	Poultry, Edible offal of	*0.005
PM 0110	Poultry meat	*0.005
ADD		
PE 0112	Eggs	T*0.0001
MO 0818	Pig, Edible offal of	T*0.0001
MM 0818	Pig meat	T*0.0001
PO 0111	Poultry, Edible offal of	T*0.0001
PM 0110	Poultry meat	T*0.0001

On completion of the phase-out period, the temporary MRLs in Table 1 of the MRL Standard are to be deleted. The amended residues definition in Table 3 of the MRL Standard will remain as a reference for testing of analytes during ongoing residues monitoring and surveillance.

10. IMPACT OF THE REGULATORY APPROACH AND AVAILABILITY OF ALTERNATIVE TREATMENTS

Table 8. Summary of the anticipated impact of the regulatory approach, and registered alternative chemicals

Disease	Impact	Alternative
Registrants		
Histomoniasis (blackhead), Swine dysentery	Disposal of unused dimetridazole after a phase-out could be costly. There is no compensation mechanism in place.	None is available.
Producers of broiler breeders and breeder turkeys		
Histomoniasis (blackhead)	Excess fertilised eggs and meat from spent hens may be marketed for human consumption during a phase-out period. There is need to observe lengthy meat and egg withholding periods. Chickens and turkeys reared on litter are at increased risk of disease. Outbreaks of blackhead after a phase-out could result in economic loss of valuable breeder poultry.	There are no alternative registered chemicals for treating blackhead, but industry could seek registration of effective anthelmintics that control caecal worms.
Producers of table egg layer flocks		
Histomoniasis (blackhead)	These producers are restrained from using dimetridazole. Management of pullets reared in cages, on litter and free range may require modification so as to reduce the chance of the disease occurring. Husbandry of sheds may need to be amended so as to control the caecal worm, which is the carrier of the causative disease agent.	There are no alternative registered chemicals for treating blackhead, but industry could seek registration of effective anthelmintics that control caecal worms.
Hobby poultry producers and others		
Histomoniasis (blackhead) Trichomoniasis (canker)	Hobby producers are restrained from using dimetridazole if they are supplying meat and eggs for human consumption. The impacts are those described for niche industries (see below).	There are no alternative registered chemicals for treating blackhead, but industry could seek registration of effective anthelmintics that control caecal worms. Carnidazole and ronidazole are available for treating canker.

Disease	Impact	Alternative
Niche Industries		
Trichomoniasis (canker)	Loss of preferred option for group treating canker in pigeons. Carnidazole is marketed for individual bird treatment but ronidazole is suitable for group treatment as it can be administered in drinking water. Neither alternative can be used in birds intended for human consumption. Ronidazole possesses mutagenic and carcinogenic properties. The restrictions that are applied to turkeys and chickens are also applied to pigeons for squab production.	Two registered alternatives, carnidazole and ronidazole are available.
Pig Producers		
Swine dysentery	There is little impact because there are several registered alternatives.	Tiamulin, tylosin, lincomycin and olaquindox are available. The APVMA has published an application summary for Aivlosin.
Consumers		
	Oral exposure to dimetridazole is reduced	Not applicable.
Workers and end users		
	Workers and users are required to wear personal protective equipment and observe safety directions so as to reduce dermal and inhalation exposure to dimetridazole during the phase-out.	Not applicable.

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[MB] = May & Baker
[RP] = Rhône-Poulenc Sante

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APPENDIX A: Toxicology hazard profile of dimetridazole

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of oral absorption	No data in laboratory animals; absorption is ≥75% in target animals (turkeys and pigs).
Distribution	No data in laboratory animals; in pigs, highest tissue concentrations (in descending order) occur in the liver, kidneys, lungs, spleen, fat and muscle.
Potential for accumulation	Not investigated.
Rate and extent of excretion	No data in laboratory animals; total excretion (urine, faeces and expired air) is rapid in turkeys (~90% within 72 h,), relatively slow in pigs (~75% in 7 days).
Metabolism	No data in laboratory animals; metabolism is extensive in turkeys, metabolism not adequately studied in pigs.
Toxicologically significant compounds (animals, plants and environment)	Parent compound; 1-methyl-5-nitroimidazol-2-yl methyl carboxylic acid (MNICA); 2-hydroxylmethyl-1-methyl-5-nitroimidazole (HMMNI); and other metabolites.

Acute toxicity	
Rat oral LD50 (mg/kg bw)	1600
Worst oral LD50 in other species	1790 in mice
Rat dermal LD50 (mg/kg bw)	No data
Worst dermal LD50 in other species	No data
Rat inhalation LC50 (mg/m3)	No data
Worst inhalation LC50 in other species	No data
Skin irritation	Appears to be non-irritant (limited data)
Eye irritation	Appears to be non-irritant (limited data)
Skin sensitisation	No data

Short-term and subchronic toxicity	
Target/critical effect	Rats: Toxic effects on testes, ovaries, stomach and heart at ≥200 mg/kg bw/d; central nervous system toxicity at 1,000 mg/kg bw/d. Dogs: central nervous system toxicity; skeletal muscle contraction; renal, liver, bone marrow and thyroid toxicity at ≥50 mg/kg bw/d po. Testicular toxicity at ≥90 mg/kg bw/d po.
Lowest relevant oral NOEL (mg/kg bw/d)	40 in a 3-month study in dogs (but data not available for re-evaluation).
Lowest relevant dermal NOEL (mg/kg bw/d)	No data
Lowest relevant inhalation No Observed Effect Concentration (mg/m3)	No data

Genotoxicity	Genotoxic in non-mammalian cells and possibly genotoxic in mammals.
Long-term toxicity and carcinogenicity	
Target/critical effect	Liver toxicity at ~0.5 mg/kg bw/d po in rats.
Lowest relevant NOEL (mg/kg bw/d)	Could not be determined because of increased mortality and liver toxicity at ~0.5 mg/kg bw/d po (lower doses not tested).
Carcinogenicity	Benign mammary tumours in rats (dose-dependent; ≥15 mg/kg bw/day po; NOEL: 3.8 mg/kg bw/d, but data not available for re-evaluation); no data in a second species.
Reproductive toxicity	
Reproduction target/critical effect	Decreased bw gains and food intake in F0 males and decreased lactation in F0 dams at ~200 mg/kg bw/d; increased mortality in F1b offspring at ~10 and 200 mg/kg bw/d (but data not available for re-evaluation).
Lowest relevant reproductive NOEL (mg/kg bw/d)	Could not be determined.
Developmental target/critical effect	Maternotoxicity: Reduced food intake and bw gains, and abortions at all doses (30-60 mg/kg bw/d). Fetotoxicity: Death or total resorptions at 60 mg/kg bw/d; slight reduction in fetal weight (dose levels not stated). [#+]
Lowest relevant developmental NOEL (mg/kg bw/d)	Could not be determined.
Delayed neurotoxicity	No data
Immunotoxicity	No data
Dermal absorption	No data

Summary	NOEL (mg/kg bw/d)	Study	Safety factor
ADI	Could not be determined	Not available	NA[§]
ARfD (mg/kg/bw)	Could not be determined	Not available	NA
Health value in drinking water	Not necessary to set a health value because dimetridazole residues should not be found in water or water catchment areas.		

[#] Evaluation by JECFA (1990), study not evaluated by OCS.

[+] No data in a second species.

[§] Not applicable

This profile for dimetridazole is developed from the OCS assessment of data submitted to the review and the OCS consideration of JECFA evaluations completed in 1990.

In addition, PACC has assessed acute toxicity studies in the rat, rabbit and guinea pig; reproductive toxicity in the pig, and metabolism studies in the rat and pig. However, the findings of these studies in Section 2 neither dispel the concern of dimetridazole's potential genotoxic carcinogenicity, nor give support for retention of its ADI.

APPENDIX B: Dimetridazole products registered in Australia

Product number	Registrant	Product name	Formulation	Registered uses
35556	Agribusiness Products Pty Ltd	Dimetridazole FG	Oral powder, premix	Swine dysentery in pigs.
38037	Adisseo Australia Pty Ltd	Emtryl Premix for Feed Medication	Oral powder, premix	Swine dysentery in pigs, Blackhead in poultry and turkeys.
38038	Adisseo Australia Pty Ltd	Emtryl Soluble Dimetridazole Soluble Powder 400g/kg	Soluble powder	Blackhead in game birds, poultry and turkeys Trichomoniasis (canker) in pigeons, Swine dysentery in pigs.
50141	Bronson and Jacobs Pty Ltd	Bronson and Jacobs Dimetridazole (D.M.Z.) Oral	Oral powder, premix	Swine dysentery in pigs, Blackhead in poultry and turkeys.
50743	Ridley Agriproducts Pty Ltd	CCD Dimetridazole (Water Soluble Powder)	Oral powder, premix	Trichomoniasis (canker) in caged birds and pigeons, Blackhead in game birds, poultry and turkeys, Swine dysentery in pigs.
52812	Ridley Agriproducts Pty Ltd	CCD DMZ 225 Premix (Dimetridazole)	Oral powder, premix	Swine dysentery in pigs, Blackhead in poultry and turkeys.

APPENDIX C: Dimetridazole product registrations to be cancelled

Product number	Product name	Registrant	Label approval numbers
35556	Dimetridazole FG	Agribusiness Products Pty Ltd	35556/01Ψ
38037	Emtryl Premix for Feed Medication	Adisseo Australia Pty Ltd	38037/0900
50141	Bronson and Jacobs Dimetridazole (D.M.Z.) Oral	Bronson and Jacobs Pty Ltd	50141/0798
52812	CCD DMZ 225 Premix (Dimetridazole)	Ridley Agriproducts Pty Ltd	52812/0801 52812/0600

Ψ Label transitioned from the states and not having an approval number.

APPENDIX D: Dimetridazole product registrations and labels to be varied

Product number	Product name	Registrant	Label approval numbers
38038	Emtryl Soluble Dimetridazole Soluble Powder 400g/kg	Adisseo Australia Pty Ltd	38038/0900
50743	CCD Dimetridazole (Water Soluble Powder)	Ridley Agriproducts Pty Ltd	50743/0201

Note: Label Approval 50743/1200, the non-current label of CCD Dimetridazole (Water Soluble Powder), is cancelled on the basis that it does not contain adequate instructions.